JOURNAL
of
THE SOUTH AFRICAN CHEMICAL INSTITUTE

1960

April
VOLUME XIII
NUMBER 1

April
DEEL XIII
NOMMER 1

Price 7/6 *Prys*

JOERNAAL
van
DIE SUID-AFRIKAANSE CHEMIESE INSTITUUT

SURFACE IMPRESSIONS AND INSIDE KNOWLEDGE

The organic chemicals manufactured by National Chemical Products Limited play their part in the industrial life of our country and contribute to an ever increasing number of products now "Made in South Africa".

Surface coatings, hydraulic brake fluids, synthetic upholstery, dissolving of acetylene, pharmaceuticals, perfumes and balanced rations are but a few of the preparations and manufactures which rely upon the exact specifications of our growing range of chemical products.

Technicians of N.C.P. are continually helping to solve the varied problems of industry by developing new products and extending the uses of existing ones. The skill and experience of N.C.P. is ever freely at your disposal.

- Absolute Alcohol
- Acetic Acid
- Acetone
- Amyl Acetate
- Amyl Alcohol
- Animal Feed Supplements
- Butyl Acetate
- Butyl Alcohol
- Carbon Dioxide Gas
- Dibutyl Phthalate
- Di-Iso-Octyl Phthalate
- Dry Ice
- Ethyl Acetate
- Froth Flotation Reagents
- Industrial Alcohol
- Methylated Spirit
- Moulding Powders
- Nitrocellulose Lacquer Thinners
- Phthalic Anhydride
- Rectified Spirit
- Vinegar

NCP

NATIONAL CHEMICAL PRODUCTS LIMITED

P.O. BOX 344 • GERMISTON • TRANSVAAL

Branches at: Cape Town, Port Elizabeth and Salisbury
(Subsidiary Companies: Umgeni Distilleries Limited, Durban
Poly-Resin Products Limited, Durban)

Die Redakteur en die Instituut aanvaar geen verantwoordelikheid vir verklarings wat gemaak word of menings wat uitgespreek word deur bydraers nie.

The Editor and the Institute assume no responsibility for statements made and opinions expressed by contributors.

Publications Committee/Publikasie-komitee

S. S. Israelstam, M.Sc., Ph.D., F.R.I.C. (*Chairman/Voorsitter*)
O. G. Backeberg, M.A., D.Sc., F.R.I.C.
S. Goodman, B.Sc. (Eng.), F.R.I.C.
A. W. Lategan, M.Sc., Ph.D.
H. A. E. Mackenzie, M.Sc., Ph.D., D.I.C., A.R.I.C.
K. Mathieson, D.Sc.
G. W. Perold, D.Sc., Dr. sc. techn.
W. S. Rapson, M.Sc., D. Phil., F.R.I.C., F.R.S.S.A.

and the Editor of the Journal/en die Redakteur van die Joernaal,
D. E. A. Rivett, M.Sc., Ph.D.

Published by the Institute, Kelvin House **Uitgegee deur die Instituut, Kelvinhuis**
Johannesburg

OFFICE BEARERS AND COUNCIL—1959-60—AMPSDRAERS EN RAAD

President:
G. W. Perold, D.Sc., Dr. sc. techn.

Immediate Past President/Vorige President:
H. A. E. Mackenzie, M.Sc., Ph.D., D.I.C., A.R.I.C.

Vice-President/Vise-President:
F. L. Warren, Ph.D., D.Sc., A.R.C.S., D.I.C., F.R.I.C.

Honorary Secretary and Treasurer/Ere-Sekretaris en Tesourier:
M. Mrost, M.Sc., F.R.I.C.

Chairman of the Publications Committee/Voorsitter van die Publikasie-Komitee:
S. S. Israelstam, M.Sc., Ph.D., F.R.I.C.

And/En
L. H. Ahrens, M.A., B.Sc., D.Sc., F.R.I.C.
O. G. Backeberg, M.A., D.Sc., F.R.I.C.
F. L. Clark, M.Sc., D.Sc.
P. R. C. Cooke, B.A., B.Sc.
S. Goodman, B.Sc. (Eng.), F.R.I.C.
F. Hawke, M.Sc. (Eng.), Ph.D., F.R.I.C., A.M.I. Chem. E.
G. S. James, O.B.E., M.Sc.
H. J. Koch, M.Sc., A.R.I.C.
A. W. Lategan, M.Sc., Ph.D.
L. J. le Roux, M.Sc., B.Com., D.Sc.
H. N. C. Maclennan, B.Sc. (Eng.), A.M.I. Chem. E., A.R.I.C.
K. Mathieson, D.Sc.
W. M. Neale-May, B.Sc. (Eng.), M.I. Chem. E., M. Inst. Pet., A.M.S.A.I. Mech. E.
A. J. Petrick, M.Sc., Dipl. Ing., Dr.Ing.
W. S. Rapson, M.Sc., D.Phil., F.R.I.C., F.R.S.S.A.
D. E. A. Rivett, M.Sc., Ph.D.
R. E. Robinson, B.Sc. (Eng.), Ph.D.
P. K. F. J. van Gent, M.Sc., D.Ing., H.E.D.

CONTENTS — INHOUD

Page/Bladsy

AN ISOTOPE DILUTION PROCEDURE FOR THE DETERMINATION OF THE TRUE PHOSPHATE CONTENT OF ROCK PHOSPHATES *by* J. RIJKHEER .. 1

THE SPECTROGRAPHIC DETERMINATION OF ZINC IN PLANT MATERIAL ASH USING THE 2138·6 Å SPECTRUM LINE *by* A. STRASHEIM AND D. J.EVE 7

GAS-VLOEISTOFVERDELINGSCHROMATOGRAFIE. DIE EFFEK VAN DIE KOLOMLENGTE OP DIE EFFEKTIWITEIT *deur* W. J. DE WET EN VICTOR PRETORIUS 13

THE GRANULAR SUPPORT IN GAS-LIQUID CHROMATOGRAPHY. THE EFFECT OF THE PARTICLE SIZE OF THE GRANULES AND OF THE EFFECTIVE THICKNESS OF THE LIQUID FILM ON THE COLUMN EFFICIENCY *by* W. J. DE WET, P. C. HAARHOFF AND VICTOR PRETORIUS 19

Cis-trans ISOMERISM OF AMINOAZO COMPOUNDS *by* J. DIJKSTRA 27

RADIOLYSIS OF FERROUS SULPHATE SOLUTIONS WITH STANDARDISED INTERNAL SOURCES OF PHOSPHOROUS-32 *by* M. PEISACH AND J. STEYN .. 34

THE BITTER PRINCIPLE OF *Solanum Melongena* (EGG PLANT) FRUITS *by* H. L. DE WAAL, L. P. NEETHLING AND G. W. PEROLD 45

COMPLEX CYANIDES OF TRIVALENT GOLD *by* P. O. FINSEN AND K. A. MURRAY 48

SOME PROPERTIES OF NITROCOTTON-NITROGLYCERINE GELS *by* P. W. LINDER 57

NOTES

STYRENE-*P*-CARBOXYLIC ACID *by* L. DYKSTERHUIS AND D. E. A. RIVETT .. 68

PRODUCTION OF FAT BY *Polyporus Rugulosus* *by* JACK ADLER.. 70

Royal
GILLET
JAV

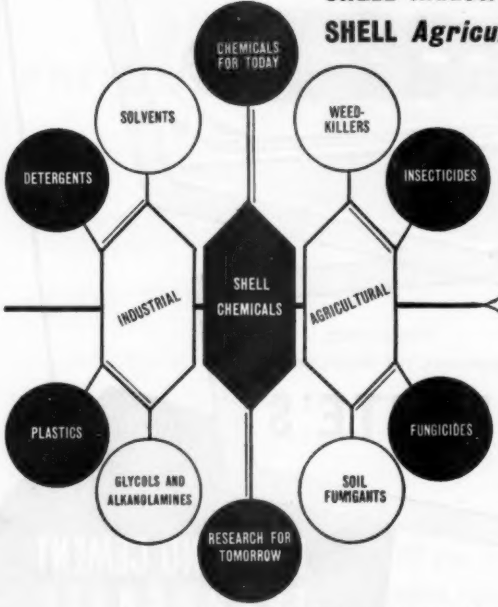
A modern state emerges as
South Africa's
farmers, engineers, miners
and manufacturers spread
their **pattern** of
achievement over the veld.
AE & CI is privileged
to serve them . . . with
research vital to
development and hundreds
of products used by the
industries contributing
to a better life and the
nation's **progress.**

AE&CI AFRICAN EXPLOSIVES AND CHEMICAL INDUSTRIES LIMITED

WK4017

FAMILY with a SHARE IN YOUR FUTURE

SHELL ***Industrial Chemicals***
SHELL ***Agricultural Chemicals***



CHEMICALS FOR TODAY
SOLVENTS
WEED-KILLERS
DETERGENTS
INSECTICIDES
INDUSTRIAL
SHELL CHEMICALS
AGRICULTURAL
PLASTICS
FUNGICIDES
GLYCOLS AND ALKANOLAMINES
SOIL FUMIGANTS
RESEARCH FOR TOMORROW

Shell Chemicals already have a share in your life. The fruit you eat, the bread you toast — even the tobacco you smoke, is grown under the protecting arm of Shell Agricultural Chemicals. Perhaps the suit you're wearing was cleaned with a Shell Chemicals detergent. Certainly you've used Shell Chemicals plastics and solvents.

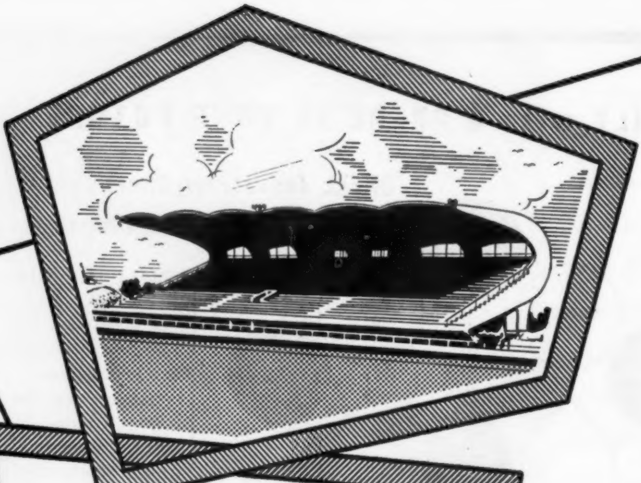
Shell Chemicals are a part of modern living — an increasingly important part as the boundaries of knowledge are pushed further back and organic chemistry provides better products, surer protection, faster, cheaper processes.

The SHELL Chemicals family has a BIG share in your future

THE SHELL CHEMICAL COMPANY OF AFRICA LIMITED
(Incorporated in England)
Chemical Partner of Agriculture and Industry
Branches at: Cape Town, Port Elizabeth, Durban, Johannesburg, Salisbury and Nairobi.

SHELL

Grant [illegible]



THIS SYMBOL
IDENTIFIES THE PRODUCTS OF

WHITE'S

A PRODUCT OF 'N PRODUK VAN WHITE'S

PORTLAND CEMENT

FERROCRETE*
THE RAPID HARDENING PORTLAND CEMENT

SNOWCEM*
THE WATERPROOF CEMENT PAINT

COLORCRETE*
COLOURED PORTLAND CEMENT

CULLAMIX*
COLOURED CEMENT & SPECIAL AGGREGATE

AQUACRETE*
THE WATER-REPELLENT CEMENT

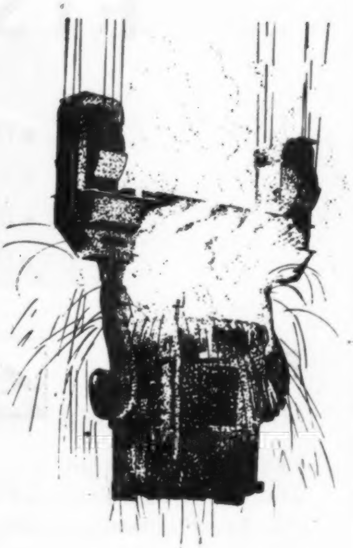
"417 CEMENT"
QUICK SETTING EXTRA RAPID HARDENING

Tyrolean* WALL FINISH
COLOURED CEMENT & SPECIAL AGGREGATE

FOR
MODERN
CONSTRUCTIONS

WHITE'S SOUTH AFRICAN
PORTLAND CEMENT CO. LTD.
(INCORPORATED IN THE UNION OF SOUTH AFRICA)
P.O. BOX 2484
JOHANNESBURG
TELEPHONE 33-7411

*REGISTERED TRADE MARKS



STEEL PRODUCTION

AIDS THE CHEMIST

By-products of the coke ovens become raw material for the chemist. His skill in the laboratory has produced a host of things such as paints and preservatives, insecticides and solvents.

In addition to supplying about 70 per cent of South Africa's steel requirements, Iscor also supplies large quantities of coke oven by-products to the chemical industry. The chemist in turn aids steel production, for Iscor chemists are responsible for quality control—a most important function in any industry—especially in making steel.

SOUTH AFRICAN IRON AND STEEL INDUSTRIAL CORPORATION, LIMITED

P.O. BOX 450 **PRETORIA**

FISONS

for

- FERTILIZERS
- WEEDICIDES
- INSECTICIDES
- FUNGICIDES

FISONS
(PTY.) LIMITED

P.O. Box 1893, Durban
P.O. Box 3, Kuils River, C.P.
P.O. Box 102, Sasolburg, O.F.S.

FISONS
PEST CONTROL
(S.A.) LIMITED

P.O. Box 1893, Durban
P.O. Box 61, Stellenbosch, C.P.

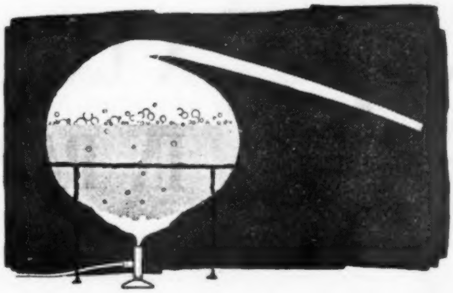
Manufacturers of the famous

AFRICAN MAID
PRODUCTS

Suppliers of Glucose, Starches, Modified Starches and Dextrines to South African and Overseas Industry. All products made from South African Maize.

AFRICAN PRODUCTS
MANUFACTURING COMPANY LIMITED

Industrial Sites (East), P.O. Box 554, Germiston



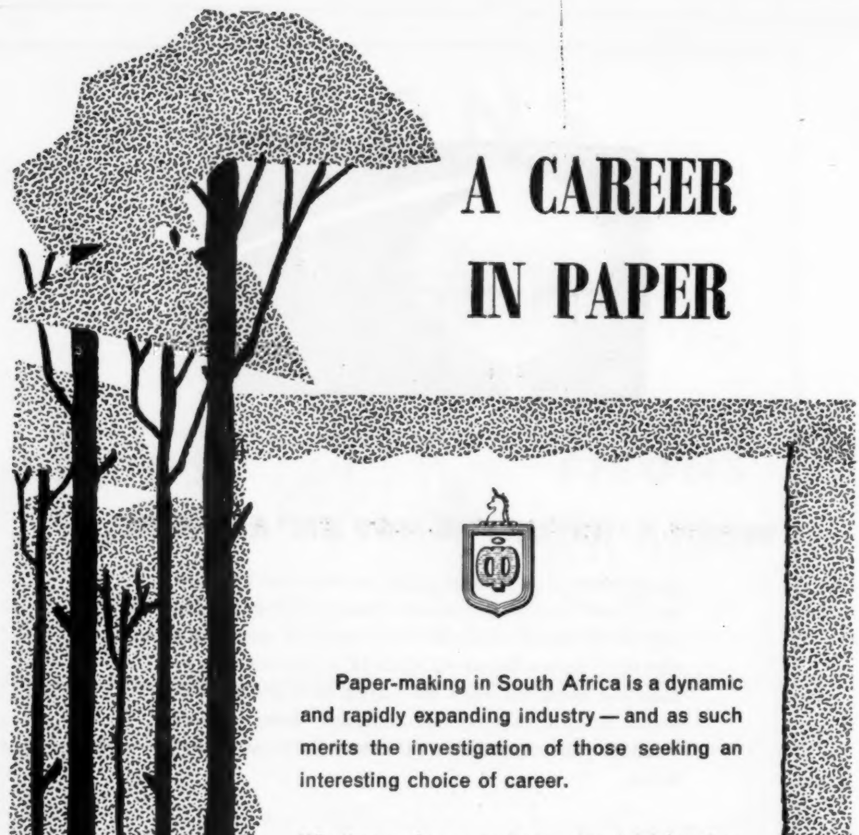
WHEN A 'RETORT' IS NOT AN 'ANSWER'...

The problems of industry are seldom so simple that the answers can be found in a retort! Extensive research facilities are necessary; so are skilled technicians with a world of experience behind them. Such is the service which I.C.I. provides. New products and techniques which are playing an important role in South African Industry are continually being developed. Whatever *your* requirements, I.C.I. is ready to be of service to you.

ICI

IMPERIAL CHEMICAL INDUSTRIES (SOUTH AFRICA) LIMITED

Imperial Chemical House, 1 Leyds St., P.O. Box 11270, Johannesburg
Sales Offices: Cape Town, Durban, Port Elizabeth, Salisbury



A CAREER IN PAPER

Paper-making in South Africa is a dynamic and rapidly expanding industry — and as such merits the investigation of those seeking an interesting choice of career.

Whether you're an engineer, chemist, laboratory assistant, accountant, clerk, apprentice or artisan, write and tell us about yourself. We at SAPPI at all times welcome enquiries from young men and women interested in making paper-making their career.

Letters should be addressed to **THE PERSONNEL OFFICER, SOUTH AFRICAN PULP AND PAPER INDUSTRIES, LTD., P.O. ENSTRA via SPRINGS, TRANSVAAL.**

SAPPI

young 4086

AN ISOTOPE DILUTION PROCEDURE FOR THE DETERMINATION OF THE TRUE PHOSPHATE CONTENT OF ROCK PHOSPHATES

by

J. RIJKHEER

OPSOMMING

Die ontwikkeling van 'n isotopiese verdunningsmetode vir die bepaling van die fosfaatinhoud van rotsfosfate met behulp van P^{32} word beskryf. Daar word aangetoon dat akkurate en presiese resultate met die metode verkrygbaar is.

SUMMARY

The development of an isotope dilution procedure, using P^{32}, for the determination of the phosphate content of phosphate rocks is described. It is demonstrated that accurate and precise results are obtainable by this method.

INTRODUCTION

The primary objective of the work described here was to develop a reference method of the highest possible accuracy for the determination of the phosphate content of rock phosphates. Since the method was not intended for routine use, ease of application, speed and economy were relatively unimportant considerations. The main emphasis was on obtaining an unbiased result, the accuracy of which was not dependent on a compensation of errors, as is usually the case in gravimetric methods, or on the suitability of volumetric procedures for work of the highest precision.

With these requirements in mind it was considered that a direct isotope dilution method, using radioactive phosphorus, was worthy of investigation. This method is only one of a number of isotope dilution procedures which have been increasingly applied in recent years in both organic and inorganic analytical work. To our knowledge the procedure is currently used in at least two reference methods in organic analyses.[1, 2]

Our preference for an isotope dilution procedure, using P^{32}, was based mainly on considerations pertaining to the great sensitivity whereby radioactive materials can be detected, the improved precision obtainable by replication of the radiometric measurements and the certainty with which the identity of the element being measured can be established. A further attraction considered was the fact that the technique does not require quantitative recovery at any stage of the analysis.

The relatively more critical demands on purity when using radioisotopes,[3] were no obstacle since radiochemical purity and identity were easily established,[4] and adequate steps for purification could be taken. Isotopic fractionation and exchange reactions of the labelling atoms of the active compound could be obviated by the experimental conditions. A suitable ratio between labelled and endogenous material, as described by Radin,[5] could readily be obtained to ensure maximum accuracy and, finally, statistical purity of the nuclear counting was readily ensured by any of a number of techniques, an excellent example of which is due to Riedel.[6]

In the search for a suitable phosphate standard, silver phosphate was initially favoured. This was, however, abandoned in favour of magnesium pyrophosphate when it was found that the latter compound could be prepared in as pure a form as silver phosphate by the relatively simple standard procedure of Hoffman and Lundell.[7] Because of the specificity of this procedure for orthophosphates, uniform isotopic dilution in the rock assay could, apart from intimate mixing in solution, be effected only by ensuring that all the labelled and non-labelled isotopes were present in the ortho form.

EXPERIMENTAL

Principle of the procedure. In the isotope dilution assay of rock phosphate, accurately weighed quantities of a standard, P^{32} labelled, pure magnesium pyrophosphate and rock sample were digested and intimately mixed in acid solution and a portion of the phosphates in the mixture isolated as pure magnesium pyrophosphate. The specific activities of the standard and isolated materials were then determined and the true amount of P_2O_5 in the rock calculated from the simple relationship

$$\frac{A}{C} = \frac{z + y}{z} \quad \ldots \quad (1)$$

where z = the weight of standard labelled $Mg_2P_2O_7$ used in the experiment

y = the weight of $Mg_2P_2O_7$ equivalent to the unknown quantity of P_2O_5 in the rock sample

A = specific activity of the standard

C = specific activity of the $Mg_2P_2O_7$ isolated from the mixture of $z+y$.

Reagents and apparatus. Since the purity of the final, ignited precipitates featuring in the assay was of the greatest importance, only the purest chemicals and doubly distilled water were used throughout in preparing reagents. Ammonia and hydrochloric acid were distilled and stored in polythene and glass-ware respectively.

The magnesia mixture, as used in the standard procedure of Hoffmann and Lundell, was made up as follows. $MgCl_2.6H_2O$(50 g) and ammonium chloride (100 g) were dissolved in water (500 ml), made slightly alkaline with ammonia and allowed to stand overnight. The solution was then filtered, acidified with 10 ml excess of 5N hydrochloric acid, diluted to 1 litre and stored in a glass stoppered bottle.

Polythene-ware only was used in operations carried out in alkaline conditions in order to minimise contamination with silica.

Ion-exchange columns used in the purification stage consisted of 1½ cm diameter columns, of about 60 milli equivalents exchange capacity, of I.R. 120 cation-exchange resin in the hydrogen form.

The nuclear counting equipment consisted of a Mullard type MX124/01 M6 halogen quenched liquid counter, a 1014A probe unit with a modified input circuit as recommended on the data sheet of the liquid counter, a Dynatron scaler, type 1009D, modified to use a dekatron register instead of the conventional post office unit and a Dynatron type P200A E.H.T. power supply.

Preparation of standard magnesium pyrophosphate. In the preparation of standard magnesium pyrophosphate, sodium dihydrogen phosphate (0·6 g) dissolved in water (125 ml) in a 400 ml polythene beaker was acidified with 5N hydrochloric acid (15 ml) and magnesia mixture (30 ml) added. Concentrated ammonia was then added dropwise with vigorous mechanical stirring until precipitation just started. Stirring was continued until the precipitate was well formed. More concentrated ammonia (10 ml) was then added dropwise with continued stirring and the precipitation and digestion allowed to continue for four hours. The precipitate was separated, washed three times with 10 ml lots of ammonia-water (1:19) by decantation, dissolved in 1N hydrochloric acid (50 ml), and re-precipitated as above in 125 ml solution in the presence of only 3 ml of magnesia mixture. The precipitate was collected on an ashless filter-paper, washed free of chlorides with dilute ammonia and finally ignited to magnesium pyrophosphate.

When the precipitate was ignited by conventional techniques, difficulties were sometimes experienced with a somewhat refractory form of magnesium pyrophosphate which dissolved only very slowly in dilute hydrochloric acid and was hence unsuitable for liquid counting. An ignition procedure which invariably yielded a readily soluble product entailed the following treatment. The filter-paper containing the precipitate was placed in a platinum crucible in a muffle furnace at room temperature and heated to 250°C in one hour. The temperature was then increased by 100° every half hour until a temperature of 1050° was attained. Usually the precipitate was at constant weight after igniting at the latter temperature for one hour. The re-precipitation was not carried out in a chilled solution as recommended by Hoffmann and Lundell since quantitative recovery was unnecessary.

All the pure magnesium pyrophosphate required in subsequent work was prepared in small batches as described above. Spectrographic analysis of this material showed only the following contaminants present in more than trace quantities:

Si 0·04—0·07%
Al $<$ 0·01 %
Fe $<$ 0·01 %

X-ray diffraction studies further showed that, under the conditions obtaining in the preparation of the standard, the aluminium and iron most probably occurred as $AlPO_4$ and $FePO_4$ respectively and not as the hydroxides as might have been expected. Because of the close relationship between the proportions of phosphorus in these compounds and in the standard, it may therefore be accepted that the effect of aluminium and iron impurities in the material was negligible. The silicon impurity was most probably present as SiO_2.

The radioactive standard magnesium pyrophosphate solution was prepared as follows. About 0·100 g of pure magnesium pyrophosphate, accurately weighed to four significant figures, was dissolved and completely hydrolysed to the ortho form in a special flask suitable for permitting withdrawal of solution by weight pipette. Dissolution was affected by heating on a steambath for 30 minutes in 5N hydrochloric acid (5 ml). To this solution was added 100 ml of carrier free P^{32}, obtained from Harwell and of guaranteed radiochemical purity,[8] in dilute hydrochloric acid of pH3 - 5. Sufficient activity was added to have a final solution which, when diluted a hundred times, gave 25,000 counts per minute by the liquid counting technique used in this work. This labelled phosphate standard solution was weighed and used for dispensing the small amounts of labelled standard required in the analyses by weight aliquots.

Reliability of the procedure. The following procedure was finally adopted.

An accurately weighed quantity of the labelled phosphate standard solution (about 1·2 g) and a solution of pure magnesium pyrophosphate (0·400 g), weighed to four significant figures and corrected for SiO_2 impurity, in 5N hydrochloric acid (5 ml) was heated on a steam bath for 30 minutes to ensure complete conversion to the orthophosphate. The solution was cooled, diluted to about 80 ml, passed through the ion-exchange column at the rate of about 10 ml per minute and the column washed with 0·015N hydrochloric acid (250 ml). The phosphate in the eluate was precipitated as magnesium ammonium phosphate, the precipitate purified by re-precipitation and finally ignited to magnesium pyrophosphate. A solution of 0·300 g (weighed to four significant figures) of this material in 5N hydrochloric acid (5 ml) was diluted to 100 ml and counted. The specific activity of the precipitate was then calculated.

This determination was usually done in quadruplicate and was accompanied by the determination of the specific activity of the labelled standard magnesium pyrophosphate. The latter determination involved the counting of five different solutions prepared from accurately weighed quantities of about 0·90 g of the standard labelled solution and solutions of 0·299 g of non-active magnesium pyrophosphate in 5N hydrochloric acid (5 ml), the total being diluted to 100 ml with water. All the solutions had practically the same density and composition and gave about the same count rate of around 20,000 c.p.m. It was therefore unnecessary to apply self-absorption corrections to the count rates obtained and errors arising from coincidence corrections were minimised.

The final dilution of all the solutions was done in the counting room where the temperature variation was no more than 2°C over the whole of the counting operation. Standard and other solutions were counted alternatively, starting and ending with a standard solution. The possibility of biassed estimates resulting from possible drifts in the characteristics of the counting equipment was thus minimised. Volume corcorrections to the solutions counted were negligibly small because of the low temperature fluctuations.

Using a stopwatch, four counts of four minutes each were normally taken on each solution. Background counts were taken between counts of solutions. The mean count rates, corrected for background, coincidence and decay (in this order), of individual solutions were then used in calculating the specific activities of the standard and isotopically diluted material. The weights of magnesium pyrophosphate used in each case were corrected for $Si0_2$ impurities. Using the mean of the five estimates of the specific activity of the standard active material and the specific activities obtained for the individual isotopically diluted specimens, the amounts of non-labelled magnesium pyrophosphate used in the experiments were calculated by the basic equation (1) above. The ratio of a calculated amount to the amount known to have been added was then taken as a measure of the reliability of the procedure.

The results initially obtained were surprisingly erratic for this apparently simple technique. It transpired that small amounts of insoluble magnesium pyrophosphate and incomplete conversion of phosphates to the ortho form in the isotopic dilution step were the main sources of error. After considerable experience, especially in the ion-exchange step, it was shown that with adequate operator skill and rigorous adherence to the procedure, the method yielded accurate results and was capable of acceptable precision as is shown in the table of results.

Isotope dilution assay of phosphate rock. A finely ground and thoroughly mixed sample of Morocco rock passing a 60 mesh B.S. sieve was used in this assay. The radioactivity of this material was ignored in the analysis since it had been found that, under the conditions of the experiment, its contribution to count rates was hardly detectable.

In applying the foregoing procedure to the assay, 1·20 g of the standard labelled solution, accurately dispensed by weight pipette, and a weighed quantity of sample, containing about 0.25 g of P_20_5, was digested with a 15:1 mixture of concentrated hydrochloric and nitric acids (16 ml) in a 250 ml Pyrex beaker on a steambath. The digest was taken to dryness on the steambath, baked at 105 to 110° for one hour, taken into solution and condensed phosphates formed in the baking step converted to the ortho form by heating for 30 minutes in 2·5N hydrochloric acid (10 ml) on a steambath. Silica was removed by filtration through a Whatman No. 40 filter paper followed by washing with six 10 ml portions of hot water. Thereafter the procedure was identical to that developed for the reliability test. Spectrographic analyses of the precipitates finally obtained in the rock analysis showed that only the following impurities were present in significant quantities:

Si 0·05—0·08%
Al $<$ 0·01 %
Fe $<$ 0·01 %

The P_20_5 content of the rock was calculated by the following modification of the basic equation, using weights of magnesium pyrophosphate which had been corrected for impurities:

$$\% \, P_20_5 = 63{\cdot}772 \, z \left(\frac{A}{C} - 1\right)/W$$

where z, A and C are as given before and W is the weight of sample used.

The following results were obtained in the reliability test and in the isotope dilution assay of the phosphate rock.

Description	Number of replications	% P_2O_5		$Mg_2P_2O_7$ $\frac{\text{calculated}}{\text{added}} \times 100$	
		$\bar{x}$	s	$\bar{x}$	s
Reliability test	20	—	—	99.97	0.34
Rock assay	28	32.97	0.14	—	—

DISCUSSION

The high precision obtained in this work may be explained by the many precautions taken and by the meticulous attention given to detail in the procedure. The reliability test can be considered as giving a valid indication of the accuracy of the assay of the rock sample, since the final answer in both cases was chemically dependent only on the purity of the material isolated.

It can be accepted that adequate mixing of labelled standard and sample had been effected. Isotopic fractionation may be considered as being practically absent and misappropriation of phosphate has been prevented by ensuring that, prior to ignition, the phosphate was always present in the ortho form. The nuclear counting, besides being of assured statistical purity, was arranged in a manner designed to minimise bias. It may therefore be accepted that the results obtained in the rock assay are accurate and precise.

The author is indebted to Messrs. African Explosives and Chemical Industries, Limited, for permission to publish this paper and Drs. H. A. E. Mackenzie, L. H. Stein and Mr. H. Marr for many useful discussions in connection with the work.

Research Department,
African Explosives and Chemical Industries, Ltd.,
P.O. Northrand,
Transvaal.

Received October, 9, 1959.

REFERENCES

[1] U.S. Pharmacopeia XV, 1956, First Supplement, p. 21.
[2] Craig, Tryon and Brown, *Anal. Chem.*, 1953, **25**, 1661.
[3] Christian *et al.*, *J. Am. Pharm. Soc.* (Scientific Edition), 1953, XL II, No. 5, 304.
[4] Wagner and Guinn, *J. Am. Chem. Soc.*, 1953, **75**, 4861.
[5] Radin, *Nucleonics*, 1947, **1**, No. 2, 48.
[6] Riedel, *ibid.*, 1954, **12**, No. 6, 64.
[7] Hoffman and Lundell, *Bur. Stds. J. Res.*, 1930, **5**, 282.
[8] Radiochemical Centre, Amersham, England, *private communication*.

THE SPECTROGRAPHIC DETERMINATION OF ZINC IN PLANT MATERIAL ASH USING THE 2138·6 Å SPECTRUM LINE

by

A. STRASHEIM and D. J. EVE

OPSOMMING

'n Metode word beskryf vir die spektrografiese ontleding van plantmaterialas vir sink deur gebruik te maak van die spektrumlyn te 2138·6 Å. Ilford ,,Q1" ultravioletgevoelige plate is gebruik om die spektrum vas te lê. 'n Stallwood-tipe lugtregter is om die boog gebruik om die moeilikheid van selfabsorpsie te oorkom. Vir dertig herontledings van een monster is 'n standaard afwyking van 9·3% bereken. 'n Reeks standaard monsters is volgens die metode ontleed en die resultate met die van ander metodes vergelyk.

SUMMARY

A method is described for the spectrographic analysis of plant material ash for zinc using the spectrum line at 2138·6 Å. Ilford "Q1" ultraviolet-sensitive plates were used for recording the spectrum. A Stallwood-type air jet was used round the arc to overcome the difficulty of self absorption. The standard deviation of the method was calculated to be 9·3% from 30 replicate analyses of one sample. A series of standard samples was analysed and the results are compared with those obtained by other methods.

INTRODUCTION

Zinc in plant materials can be determined spectrographically either directly on the plant ash[1, 2, 3, 4], or after carrying out a preliminary chemical separation and concentration of this element[5, 6].

The direct determination of zinc in the plant ash has certain advantages such as:

(1) less manipulation of the sample compared to the concentration methods and thus the possibility of loss of zinc is diminished;
(2) shorter lapse of time before results are available;
(3) larger volume of samples, which is of value in many fruit culture problems where only zinc is of importance.

Most spectrographic methods based on analysis of the ash, without a preliminary chemical separation, were reported in the literature more than a decade ago. Since then the spectrochemical analytical technique has improved considerably and new types of photographic plates for the far ultraviolet have become available. A critical re-evaluation of the possibilities of the spectrographic method for the determination of zinc in plant materials using the line at 2138·6 Å thus seems long overdue.

Rogers and Gall[2] found that, with the mean of five arcings taken for each result, their probable error varied from 3% to 17% and they attributed the error to one or more of five possible causes, viz.:

(1) contamination from the internal standard or from the graphite electrodes;
(2) non-uniform distribution of the internal standard throughout the sample;
(3) variation of exposure conditions (wandering of the arc, change of line voltage, etc.);

(4) influence of varying major constituents of the ash on volatility and "excitability" of the zinc atoms;

(5) photometric errors.

O'Connor[3] used the 2138 Å line in his analysis of fertilisers for zinc. He carried our eight replicate analyses to test the precision of the method and the percentage standard deviation calculated from these results is 11·7%.

Eastmond and Williams[4] found that self absorption caused errors, particularly at higher concentrations, and recommended the use of line-width measurements for determining the zinc content. The percentage standard deviation of their methods was given as 10-14%.

In the method described in this paper the precision has been improved by overcoming to some extent the errors caused by self absorption, variation of source conditions and variation of the major constituents.

EXPERIMENTAL

Spectrograph. Hilger Large Littrow with quartz optical system.

Source unit. Constant current source unit[7].

External optics. As it was found that the quartz lenses normally used in the external optical system of the spectrograph absorbed a large proportion of the radiation between 2100 and 2200 Å, these were replaced by a single "suprasil" lens (high transmittancy down to 1800 Å) which focussed an image of the source on to the collimator of the spectrograph. This lens was placed so that an image of the central 4 mm of the arc column filled the 5 cm collimator aperture which was surrounded by nonreflective material.

Plates. Ordinary photographic emulsions are not very sensitive to radiation below 2500 Å. To overcome this difficulty experiments were carried out with various types of plates and a number of ultraviolet sensitizing agents. It was found that, while these materials gave increased sensitivity in the ultraviolet, they often gave rise to "patchy" spectrograms which were unsuitable for quantitative density measurements. Experiments with thin emulsion plates proved more successful and Ilford "Q1" plates were finally selected as being the most suitable. These were cut into pieces 2 inches by 4 inches, one being used for each exposure with its centre at about 2140 Å. The plates were developed at 18° C for 6½ minutes in 1:1 I.D. 13 and fixed in Kodak X-ray fixer. Development was stopped in a stop bath of 5% acetic acid.

Excitation conditions. By using a constant current source[7] variations in emission due to current fluctuations were reduced to a minimum. Anode excitation was used and the arc burned at 10 amps.

To reduce background and increase sensitivity a relatively wide and shallow sample cavity in the anode was used. The sample electrode consisted of a 4·7 mm deep 4·5 mm diameter cavity cut into a 6·35 mm (¼ inch) diameter graphite rod. The electrode was undercut to a depth of 1 mm at a distance of 6 mm from the top.

An undercut carbon counter-electrode as described by Strasheim and Keddy[8] served as cathode.

It was evident from the slope and curvature of the first standard curves obtained that self-absorption was affecting the results (Fig. 1). Some improvement was obtained by using a deeper narrower sample electrode (8.0 mm deep, 2 mm diameter cavity) but the spectrum density was inconveniently low even when two electrodes were burned at each plate position. As shown in Fig. 1, however, the effect of self-absorption was greatly reduced by blowing an air stream upwards around the sample electrode. This had the effect of removing some of the absorbing vapour from the outer portion of the arc column. An 8 mm wide air jet of the type described by Stallwood[9] was used. In addition to reducing the influence of self absorption this was found to give a much steadier arc. The excitation conditions finally selected are given in Table I.

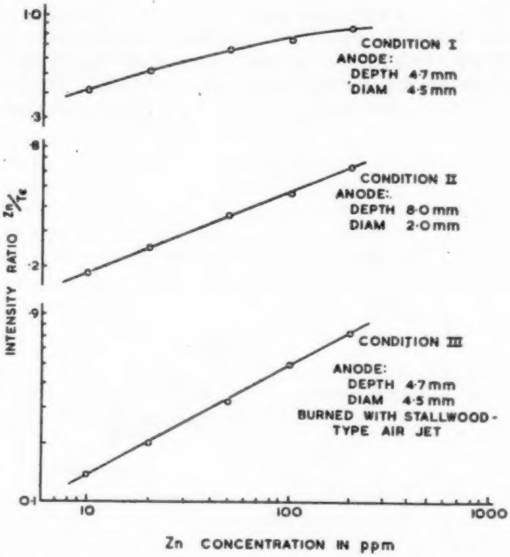


FIG. 1.—Standard calibration curves for zinc obtained under different excitation conditions

TABLE I

Standard Excitation Conditions

Light source	Positive direct current arc of 10 amp at 230 volts (constant current unit supplying the E.M.F.)
Upper electrode	Carbon electrode of 5 mm diameter with undercut rounded end.
Sample eletrode	Graphite electrode of 6·35 mm diameter. The electrode crates were 4·7 mm deep and 4·5 mm in diameter. The electrodes were also undercut to a depth of 1 mm at a distance of 6 mm from the top.
Jet	A jet with an 8 mm orifice was placed 1 mm below the undercut of the sample-holding electrode.
Electrode gap	A gap of 8 mm was maintained throughout the exposure. The central 4 mm portion of this gap was photographed.

(4) influence of varying major constituents of the ash on volatility and "excitability" of the zinc atoms;

(5) photometric errors.

O'Connor[3] used the 2138 Å line in his analysis of fertilisers for zinc. He carried our eight replicate analyses to test the precision of the method and the percentage standard deviation calculated from these results is 11·7%.

Eastmond and Williams[4] found that self absorption caused errors, particularly at higher concentrations, and recommended the use of line-width measurements for determining the zinc content. The percentage standard deviation of their methods was given as 10-14%.

In the method described in this paper the precision has been improved by overcoming to some extent the errors caused by self absorption, variation of source conditions and variation of the major constituents.

EXPERIMENTAL

Spectrograph. Hilger Large Littrow with quartz optical system.

Source unit. Constant current source unit[7].

External optics. As it was found that the quartz lenses normally used in the external optical system of the spectrograph absorbed a large proportion of the radiation between 2100 and 2200 Å, these were replaced by a single "suprasil" lens (high transmittancy down to 1800 Å) which focussed an image of the source on to the collimator of the spectrograph. This lens was placed so that an image of the central 4 mm of the arc column filled the 5 cm collimator aperture which was surrounded by nonreflective material.

Plates. Ordinary photographic emulsions are not very sensitive to radiation below 2500 Å. To overcome this difficulty experiments were carried out with various types of plates and a number of ultraviolet sensitizing agents. It was found that, while these materials gave increased sensitivity in the ultraviolet, they often gave rise to "patchy" spectrograms which were unsuitable for quantitative density measurements. Experiments with thin emulsion plates proved more successful and Ilford "Q1" plates were finally selected as being the most suitable. These were cut into pieces 2 inches by 4 inches, one being used for each exposure with its centre at about 2140 Å. The plates were developed at 18° C for 6½ minutes in 1:1 I.D. 13 and fixed in Kodak X-ray fixer. Development was stopped in a stop bath of 5% acetic acid.

Excitation conditions. By using a constant current source[7] variations in emission due to current fluctuations were reduced to a minimum. Anode excitation was used and the arc burned at 10 amps.

To reduce background and increase sensitivity a relatively wide and shallow sample cavity in the anode was used. The sample electrode consisted of a 4·7 mm deep 4·5 mm diameter cavity cut into a 6·35 mm (¼ inch) diameter graphite rod. The electrode was undercut to a depth of 1 mm at a distance of 6 mm from the top.

An undercut carbon counter-electrode as described by Strasheim and Keddy[8] served as cathode.

It was evident from the slope and curvature of the first standard curves obtained that self-absorption was affecting the results (Fig. 1). Some improvement was obtained by using a deeper narrower sample electrode (8.0 mm deep, 2 mm diameter cavity) but the spectrum density was inconveniently low even when two electrodes were burned at each plate position. As shown in Fig. 1, however, the effect of self-absorption was greatly reduced by blowing an air stream upwards around the sample electrode. This had the effect of removing some of the absorbing vapour from the outer portion of the arc column. An 8 mm wide air jet of the type described by Stallwood[9] was used. In addition to reducing the influence of self absorption this was found to give a much steadier arc. The excitation conditions finally selected are given in Table I.

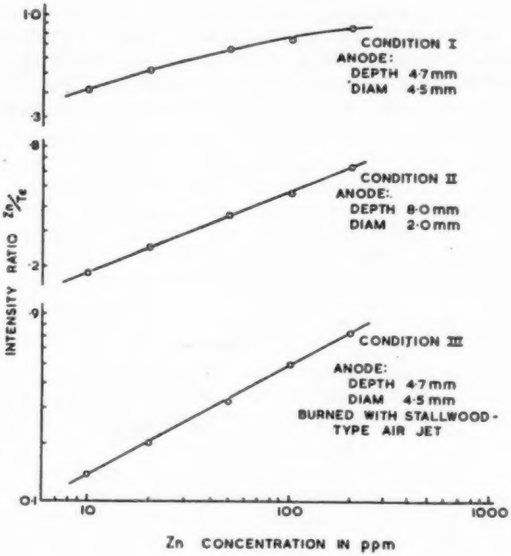


FIG. 1.—Standard calibration curves for zinc obtained under different excitation conditions

TABLE I

Standard Excitation Conditions

Light source	Positive direct current arc of 10 amp at 230 volts (constant current unit supplying the E.M.F.)
Upper electrode	Carbon electrode of 5 mm diameter with undercut rounded end.
Sample eletrode	Graphite electrode of 6·35 mm diameter. The electrode crates were 4·7 mm deep and 4·5 mm in diameter. The electrodes were also undercut to a depth of 1 mm at a distance of 6 mm from the top.
Jet	A jet with an 8 mm orifice was placed 1 mm below the undercut of the sample-holding electrode.
Electrode gap	A gap of 8 mm was maintained throughout the exposure. The central 4 mm portion of this gap was photographed.

Buffer mixture. The mixture of potassium sulphate, calcium carbonate and graphite was selected after experiments had been carried out with a number of different mixtures. A 1:10 potassium sulphate, graphite mixture was found to give a good line to back-ground ratio, but was found to give inconsistent results. A mixture of 55%calcium carbonate in graphite was used with some success, but a mixture of 10% potassium sulphate and 40% calcium carbonate in graphite was found to give a more constant emission (Fig. 2). By diluting the sample 1:9 with this buffer mixture the influence of variation in the major constituents of the sample was greatly reduced.

Internal standard. Tellurium, previously used by Rogers[1], was selected as internal standard element. It has a strong spectrum line (2142·8 Å) close to the zinc line, is unlikely to occur in plant materials and it has suitable physical properties. (Excitation potential: Zn 2138·6 Å, 5·8 eV; Te 2142·8 Å, 5·8 eV. Ionisation potential: Zn 9·392 eV, Te 9.007 eV). In addition, as can be seen from Fig. 2, the vaporisation rates of tellurium and zinc are similar under the arc conditions. One per cent of tellurium oxide was added to the buffer mixture to give a suitable constant concentration of tellurium.

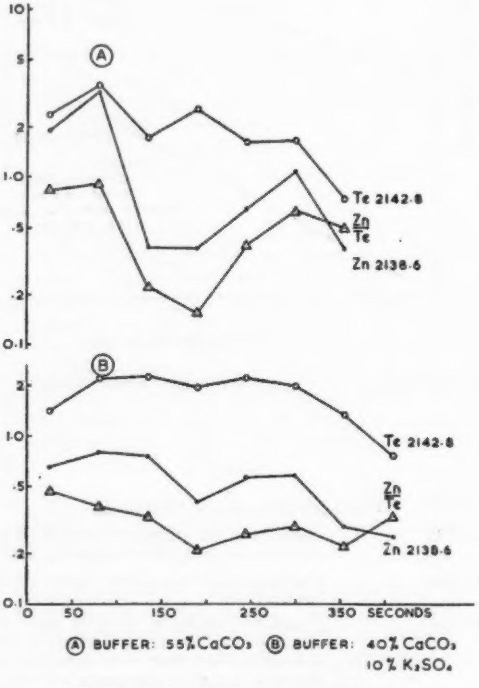


FIG. 2.—Time/intensity curves for:
(1) Zn 2138·6 Å
(2) Te 2142·8 Å
(3) Zn 2138·6 Å/Te 2142·8 Å (intensity ratio)

Plate calibration and calculation of intensity ratios. The spectrum lines were observed to be unevenly illuminated. This was attributed to some optical imperfection of the spectrograph becoming more marked at low wavelengths. For this reason no step sector or filter was used while recording the sample spectra and the mean of five transmission readings was taken for each line. For the emulsion calibration a number of copper spectra, obtained by burning a globule arc for varying periods with a two-step sector, were used to give a wide range of densities for the emulsion calibration.

An A.R.L. calculating board fitted with seidel scales was used for the calculation of intensity ratios according to the preliminary curve method described by Churchill[10].

Standards. Standards were prepared from "Specpure" zinc oxide and S.P.2 graphite to give concentrations ranging from 10 to 200 p.p.m. These standards were then mixed with the buffer-internal standard mixture in the same proportion as the samples.

Procedure. About one gram of plant material was weighed into a clean platinum crucible and ashed overnight at 450°C. The ash factor (ratio of ash: dry plant material) was determined by reweighing the crucible after ashing. The ash was well mixed and a suitable portion diluted 1:9 with the buffer. The diluted sample was then loaded into three electrodes and burned in the d.c. arc with air jet. The exposure was made for 5¾ minutes from the striking of the arc. Two or three copper spectra, obtained from a globule arc burned for different periods with a two step rotating sector, were recorded on the same plate.

RESULTS

To tests the precision of the method 10 grams of plant material were ashed and the ash, after mixing with buffer, loaded into 30 electrodes which were then burned according to the method described. The results of this series of analyses are given in Table II.

Nine standard plant samples, which had been analysed a number of times previously by chemical and spectrographic methods by the Macaulay Institute for Soil Research, by the Division of Chemical Services of the South African Department of Agriculture and in the C.S.I.R., were analysed using the method described. The results are shown in Table III.

TABLE II

Results of repeated arcing of plant ash

Arcing No.	Zn p.p.m.	Arcing No.	Zn p.p.m.	Arcing No.	Zn p.p.m.
1	67·3	11	54·4	21	52·3
2	68·1	12	51·8	22	57·3
3	63·3	13	54·0	23	60·5
4	66·4	14	54·7	24	69·6
5	54·5	15	51·1	25	58·9
6	53·1	16	58·5	26	63·3
7	55·5	17	54·5	27	54·5
8	68·1	18	59·8	28	63·7
9	57·8	19	58·5	29	60·8
10	65·1	20	62·4	30	54·5

Mean = 59·1 p.p.m.
Std. deviation = 5·48 p.p.m.
% Std. deviation = 9·3%.

TABLE III

*Results of analysis of standard samples**

Sample		4a	4b	4c	4d	4e	4g	4h	4j	4k
Analysis using present method		19·2	60·5	17·1	59·4	37·9	21·3	26·9	75·1	40·6
		19·4	53·2	17·1	65·5	36·4	22·5	26·9	69·1	45·5
		19·4	57·8	18·7	59·4	40·4	23·3	23·2	72·4	47·0
Mean		19·3	57·2	17·6	61·4	38·2	22·4	25·7	72·2	44·4
Results obtained by other methods	C.S.I.R. anode	28	60	22	59	64	25	28	48	27
	C.S.I.R. cathode	22	60	18	39	93	28	28	76	28
	Macaulay Inst.	21	60	30	60	52	26	50	45	40
	Chem. Services	26	56	23	38	30	41	43	60	18
	Mean	24	59	23	49	60	30	37	57	28

*For particulars of samples see Strasheim and Camerer[11].

CONCLUSIONS

The method described is simple and, apart from the time required for the ashing, relatively fast. It should be particularly suited for use in a laboratory where other elements are determined spectrographically after ashing the sample[8].

From the standard deviation obtained for single exposures it can be expected that the error of an analysis in which the mean of three exposures is taken would be about 5·4%, which is quite good enough for trace element nutritional studies.

The authors wish to acknowledge their indebtedness to Miss G. Behrman and Miss E. C. Maskew for their assistance with the practical work. They are also indebted to the Department of Agriculture and the Macaulay Institute for Soil Science for their analyses of the plant samples.

National Physical Research Laboratory,
South African Council for Scientific and Industrial Research,
Pretoria.

Received October 9, 1959.

REFERENCES

[1] Rogers, *Ind. Eng. Chem. Anal.*, 1935, **7**, 421.
[2] Rogers and Gall, *ibid.*, 1937, **9**, 42.
[3] O'Connor, *ibid.*, 1941, **13**, 597.
[4] Eastmond and Williams, *J. Opt. Soc. Amer.*, 1948, **38**, 666, (A.41)/801.
[5] Mitchel and Scott, *J. Soc. Chem. Ind.*, 1947, **66**, 330.
[6] Scharrer and Judel, *Z. anal. Chem.*, 1957, **156**, 340.
[7] Van der Walt, *Rev. Sci. Instr.*, 1956, **27**, 157.
[8] Strasheim and Keddy, *J.S. African Chem. Inst.*, 1958, 11, 21.
[9] Stallwood, *J. Opt. Soc. Amer.*, 1954, **44**, 171.
[10] Churchill, *Ind. Eng. Chem. Anal.*, 1944, **16**, 653.
[11] Strasheim and Camerer, *J.S. African Chem. Inst.*, 1955, **8**, 28.

GAS-VLOEISTOFVERDELINGSCHROMATOGRAFIE. DIE EFFEK VAN DIE KOLOMLENGTE OP DIE EFFEKTIWITEIT

deur

W. J. DE WET* en VICTOR PRETORIUS

SUMMARY

The van Deemter equation has been modified to include the effect of the compressibility of the carrier gas. It is shown that for each set of values of the column parameters there is a value of the pressure at the column inlet for which the column efficiency is a maximum. Under such conditions the height equivalent per theoretical plate of a column decreases with an increase in the column length.

OPSOMMING

Die van Deemter vergelyking is uitgebrei om die effek van die saampersbaarheid van die draergas in te sluit. Daar word aangetoon dat daar vir elke stel waardes van die kolomparameters 'n waarde van die gasdruk by die inlaat bestaan waarvoor die kolomeffektiwiteit 'n maksimum is. Onder hierdie omstandighede verminder die hoogte-ekwivalent per teoretiese plaat met 'n toename in die kolomlengte.

BESPREKING

Van Deemter, *et al.*[1] het aangetoon dat die effektiwiteit van 'n gas-vloeistofverdelingschromatografiekolom beheer word deur drie afsonderlike prosesse, nl. die dwarreldiffusie van die opgeloste stof as gevolg van die kronkelende gaskanale, molekulêre diffusie van die opgeloste stof in die gasfase, en die weerstand wat die opgeloste stof bied teen massavervoer.

In terme van die verskillende kolomparameters word die kolomeffektiwiteit gegee deur

$$(\mathrm{HETP})_o = 2\lambda d_p + 2\gamma D_g/U + 8(KF_g/F_l)d_f^2U/\pi^2(1+KF_g/F_l)^2D_l \quad \ldots\ \ldots\ \ldots \quad (1)$$

waar

$(\mathrm{HETP})_o$ = hoogte-ekwivalent per teoretiese plaat vir baie klein hoeveelhede opgeloste stof

γ = dwarreldiffusiefaktor

d_p = gemiddelde deursnit van die vastefasekorrels

K = distribusiekwosient van opgeloste stof tussen gas-en vloeistoffases

D_g = diffusiwiteit van opgeloste stof in gasfase

F_g, F_l = deursneë van kolom beslaan deur gas- en vloeistoffases respektiewelik

d_f = effektiewe dikte van vloeistoflagie

U = liniere snelheid van draergas

D_l = diffusiwiteit van opgeloste stof in vloeistoffase

λ = 'n konstante wat afhang van die manier waarop die kolom verpak is.

In die afleiding van hierdie vergelyking word daar aanvaar dat die draergas nie saampersbaar is nie, dat die liniêre gasnelheid dus konstant is, en dat die diffusiwiteit van die opgeloste stof geneem kan word as die gemiddelde van die waardes by die kolominlaat en-uitlaat. Vir relatief kort, losgepakte kolomme sal hierdie aanname redelik geldig wees, maar by lang kolomme, waar die drukgradient noodwendig hoog is, moet die saampersbaarheid van die draergas, en gevolglik ook die die gradient in die liniêre gasnelheid, in aanmerking geneem word.

Vergelyking (1) behoort dus alleen gebruik te word vir 'n oneindige kort gedeelte van die kolom as volg

$$(\mathrm{HETP})_x = 2\lambda d_p + 2\gamma D_{g,x}/U_x + 8(KF_g/F_l)d_f^2 U_x/\pi^2(1+KF_g/F_l)^2 D_l \quad \ldots \quad \ldots \qquad (2)$$

Die onderskrif x word gebruik om die waarde van die drukafhanklike parameters, op 'n afstand x van die inlaat, aan te toon. Die gemiddelde effektiwiteit van 'n kolom van lengte l word dan gegee deur

$$(\overline{\mathrm{HETP}})_o = \int_o^l (\mathrm{HETP})_x dx \Big/ \int_o^l dx \quad \ldots \qquad (3)$$

Dit is gerieflik om Dg,x en U_x uit te druk as funksies van die kolomlengte en van die kolomdrukke. As P_i, P_x, P_o en U_i, U_x, U_o die gasdrukke en liniêre gassnelhede by die kolominlaat is, op 'n afstand x van die inlaat, en by die uitlaat, dan is (2)

$$P_x U_x = P_o U_o = -(\beta/\eta)\, P_x \partial P_x/\partial_x \quad \ldots \qquad (4)$$

en

$$P_o U_o l = (\beta/2\eta)(P_i^2 - P_o^2) \quad \ldots \qquad (5)$$

waar β = permeabiliteitskonstante

η = viskositeit van die draergas.

Die diffusiwiteit van die opgeloste stof in die gasfase is omgekeerd eweredig met die gasdruk[3] sodat

$$D_{g,x} P_x = D_{g,o} P_o = g \quad \ldots \qquad (6)$$

waar $D_{g,o}$ = diffusiwiteit van die opgeloste stof in die gasfase by die uitlaatdruk

g = konstante, die waarde waarvan alleen afhang van die tipe opgeloste stof en die tipe draergas.

Van vergelykings (3), (4), (5) en (6) volg

$$(\mathrm{HETP})_o = 2\lambda d_p + 4\gamma g \eta l/\beta(P_i^2 - P_o^2) + 8(KF_g/F_l)d_f^2 \beta(P_i - P_o)/\eta l \pi^2 (1+KF_g/F_l)^2 D_l \qquad (7)$$

Vergelyking (7) toon aan dat vir elke stel waardes van

λ, d_p, γ, β, K, F_g/F_l, d_f, g, η, D_l, l en P_o

sal daar 'n waarde van die inlaatdruk, P_i, wees, waarvoor die gemiddelde hoogte-ekwivalent per teoretiese plaat 'n minimum sal wees. In die praktyk egter is al die parameters behalwe P_o, P_l en l, gedeeltelik onderling afhanklik, d.w.s. as die waarde van een verander word sal die waarde van een of meer van die ander ook beinvloed wees. Aangesien die verbande tussen hierdie parameters gewoonlik óf ingewikkeld óf onbekend is, sal slegs een stel waardes, wat hieronder aangegee word, in hierdie bespreking beskou word.

$2\lambda d_p = 0{\cdot}05$ cm $\qquad (4\gamma g \eta/\beta) = 66{\cdot}67 g^2 cm^2$ sek^4

$8(KF_g/F_l)d_f^2 \beta/\eta \pi^2 (1+KF_g/F_l)^2 D_l = 3{\cdot}10^{-4} g^{-1}$ cm^3 sek^2

Alhoewel hierdie waardes denkbeeldig is, kan hulle wel in die praktyk voorkom[3]. Vergelyking (7) reduseer dan na

$$(\overline{\mathrm{HETP}})_o = 0{\cdot}05 + 6{\cdot}59 \times 10^{-5} l/(P_i^2 - P_o^2) + 303{\cdot}24(P_i - P_o)/l \quad \ldots \qquad (8)$$

vir drukke in atmosfere.

Die minimum waarde van hierdie funksie word gegee deur

$$\left\{\partial(\overline{\mathrm{HETP}})_o/\partial P_i\right\} = -13{\cdot}18 \times 10^{-5} l P_i/(P_i^2 - P_o^2)^2 + 303{\cdot}24/l = 0 \quad \ldots \qquad (9)$$

Hierdie vergelyking is grafies opgelos vir verskillende waardes van die uitlaatdruk, P_o, en die kolom lengte, l. Die optimum waardes van die inlaatdruk wat op hierdie wyse verkry is, is gestip teen die ooreenstemende kolomlengte (Fig. 1). Deur substitusie van die optimum inlaatdrukke in vergelyking (8) is die minimum gemiddelde hoogte-ekwivalent per teoretiese plaat bereken en hierdie waarde is gestip teen die kolomlengte (Fig. 2). In Fig. 3 is die minimum hoogte-ekwivalent per teoretiese plaat gestel teen die uitlaatdruk. 'n Alternatiewe metode om die optimum inlaat druk te verkry is om die hoogte-ekwivalent per teoretiese plaat te stip teen die inlaatdruk volgens vergelyking (8) vir verskillende waardes van P_o en l (Fig. 4 en 5).

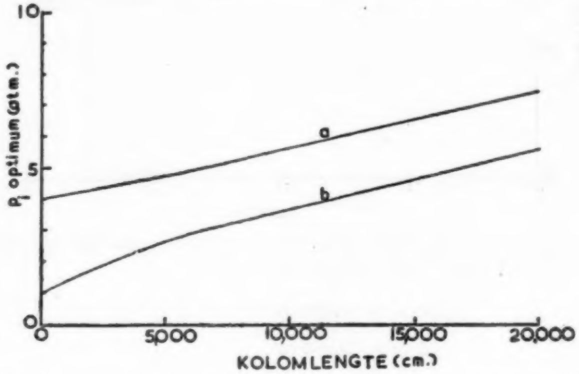


FIG. 1.—Verandering van die optimum inlaatdruk met kolomlengte
a : $P_o = 4$ atm
b : $P_o = 1$ atm

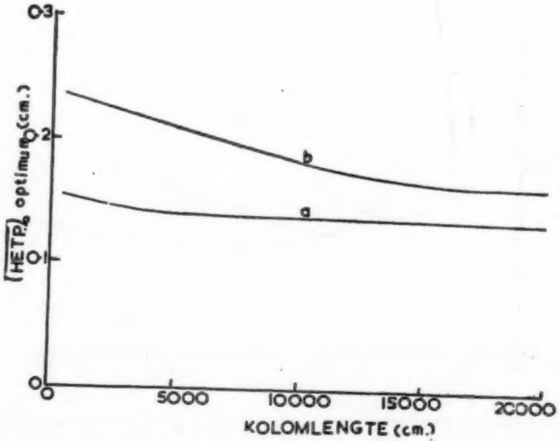


FIG. 2.—Verandering van die minimum gemiddelde hoogte-ekwivalent per teoretiese plaat met kolomlengte
a : $P_o = 4$ atm
b : $P_o = 1$ atm

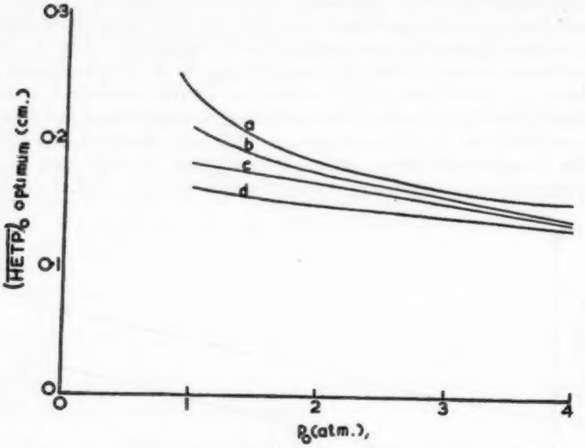


FIG. 3.—Verandering van die minimum gemiddelde hoogte-ekwivalent per teoretiese plaat met uitlaatdruk vir kolomme met verskillende lengtes

a : l = 500 cm
b : l = 5,000 cm
c : l = 10,000 cm
d : l = 20,000 cm

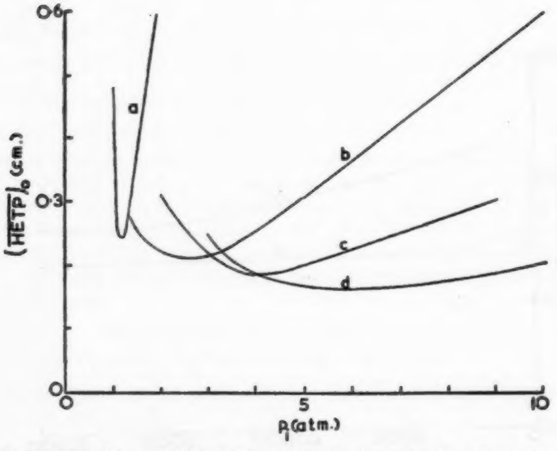


FIG. 4.—Verandering van die gemiddelde hoogte-ekwivalent per teoretiese plaat met inlaatdruk vir P_o = 1 atm

a : l = 500 cm
b : l = 5,000 cm
c : l = 10,000 cm
d : l = 20,000 cm

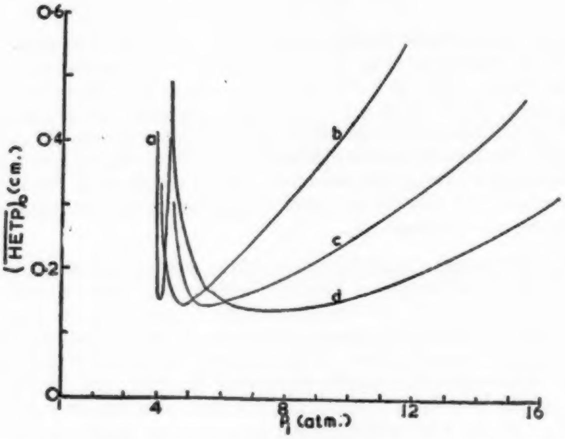


FIG. 5.—Verandering van die gemiddelde hoogte-ekwivalent per teoretiese plaat met inlaatdruk vir $P_0 = 4$ atm

a : $l =$ 500 cm
b : $l =$ 5,000 cm
c : $l =$ 10,000 cm
d : $l =$ 20,000 cm

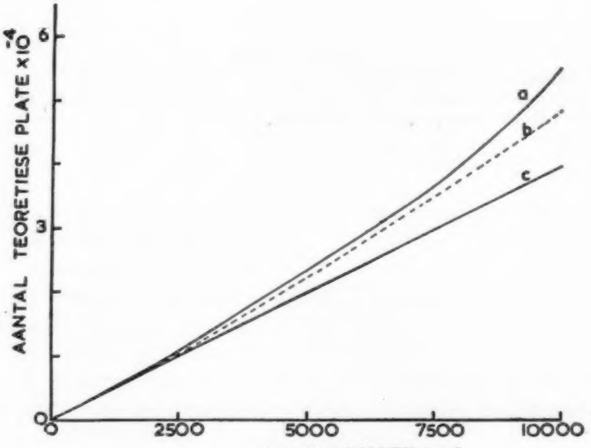


FIG. 6.—Verandering van die aantal teoretiese plate met kolomlengte

a : U_0 bereken volgens vgl (9)
b : U_0 bereken volgens onverbeterde vgl (8) vir 'n kort kolom en gebruik volgens vgl (9) vir die langer kolomme
c : U_0 bereken volgens onverbeterde vgl (8)

BESPREKING

Die minimum gemiddelde hoogte-ekwivalent per teoretiese plaat neem af met 'n toename in die kolomlengte (Fig. 2), maar die snelheid van afname is groter by lae as by hoe uitlaatdrukke (Fig. 3) totdat die kolomlengte baie groot word en dan speel die uitlaatdruk nie meer 'n rol nie. By kort kolomme moet die inlaatdruk noukeurig beheer word (Fig. 4). Aangesien die minimum van die kromme skerp is sal 'n klein afwyking van die inlaatdruk van die optimum waarde 'n relatief groot toename in die gemiddelde hoogte-ekwivalent per teoretiese plaat veroorsaak. By langer kolomme egter word die minimum platter en klein veranderings in die inlaatdruk affekteer die effektiwiteit nie so merkbaar nie.

In Fig. 6 is die totale aantal teoretiese plate gestip teen die kolomlengte. Drie krommes word gegee. Die eerste is bereken volgens die onverbeterde van Deemter vergelyking (vergelyking (1)), die tweede volgens vergelyking (8) waar die optimum inlaatdruk by elke kolomlengte gebruik word, en die derde volgens vergelyking (8) waar die optimum U_0 bepaal is met 'n kort kolom en dieselfde waarde dan gebruik is vir die langer kolomme. Uit hierdie werk volg dit dat, mits by elke kolomlengte die inlaatdruk gekies is sodat die gemiddelde hoogte-ekwivalent per teoretiese plaat 'n minimum is, sal die kolomeffektiwiteit meer as verdubbel as die kolomlengte verdubbel word.

Een van ons (V.P.) bedank "African Explosives & Chemical Industries Ltd.," vir die toekenning van 'n "Research Fellowship," asook die Wetenskaplike Nywerheidsnavorsingsraad vir geldelike ondersteuning.

Department Fisiese Chemie,
Universiteit van Pretoria.
*Tabak Navorsingsingsinstituut,
Kroondal.

Ontvang 15 *September*, 1959.

VERWYSINGS

[1] van Deemter, Zuiderweg en Klinkenberg, *Chem. Eng. Sci.*, **5**, 271 (1956).
[2] Keulemans, "*Gas Chromatography*," Reinhold Publishing Corp., N.Y., 1957, bl. 132.
[3] de Wet, Haarhoff en Pretorius, *volgende publikasie*.

THE GRANULAR SUPPORT IN GAS-LIQUID CHROMATOGRAPHY. THE EFFECT OF THE PARTICLE SIZE OF THE GRANULES AND OF THE EFFECTIVE THICKNESS OF THE LIQUID FILM ON THE COLUMN EFFICIENCY.

by

W. J. DE WET,† P. C. HAARHOFF* and VICTOR PRETORIUS

OPSOMMING

Die effek van die deursnit van die vastefase korrels en van die dikte van die vloeistoflaag op die kolomeffektiwiteit is teoreties bereken vir nie-poreuse sfere. Die gevolgtrekkings wat gemaak is, is vergelyk met eksperimentele data wat verkry is met poreuse korrels. Dit blyk dat hoër effektiwiteite verkry kan word met nie-poreuse as met poreuse korrels.

SUMMARY

The effect of the diameter of the granular support and of the thickness of the liquid film on the column efficiency has been theoretically calculated for non-porous spheres. The conclusions which have been made have been compared with experimental results which have been obtained using porous granules. It appears that higher column efficiencies may be achieved by using non-porous than porous granules.

A description of the way in which the efficiency of a gas-liquid chromatography column is affected by the particle size of the granular support and by the "effective" thickness of the liquid film is embodied in the "rate" theory developed by van Deemter, *et al.*[1] The equation derived by these authors has been modified by Pretorius, *et al.*[2] to account for the compressibility of the carrier gas. Provided the ratio of the gas pressures at the column inlet and outlet is less than approximately 2 the column efficiency may be described in terms of the various column parameters by

$$(\overline{\mathrm{HETP}})_o = 2\lambda d_p + \frac{2\gamma D_{g,o}}{U_o} + \frac{16K\left(\frac{F_g}{F_l}\right)d_f^2}{\pi^2\left(1+K\frac{F_g}{F_l}\right)^2 D_l} \cdot \frac{P_o U_o}{P_i+P_o} \quad \text{.. .. .. ..} \quad (1)$$

where $(\overline{\mathrm{HETP}})_o$ =mean height equivalent per theoretical plate for small samples

λ =a packing factor

γ =a tortuosity factor

d_p =average diameter of granular particles

$D_{g,o}$ =diffusivity of solute in gas phase at pressure at column outlet

U_o =linear gas velocity at column outlet

K =distribution coefficient of solute between gas and liquid phases

F_g & F_l =cross-sectional area of column occupied by gas and liquid phases respectively

d_f ="effective" thickness of liquid film

P_i & P_o =gas pressures at column inlet and outlet respectively

D_l =diffusivity of solute in liquid phase.

Equation (1) is sufficiently accurate for the present purpose. Since[3]

$$U_o P_o l = \epsilon^3 d_p^2 (P_i^2 - P_o^2)/360(1-\epsilon)^2 \eta \quad \text{.. .. ..} \quad (2)$$

where l =column length
ϵ =porosity of packing
η =viscosity of gas
equation (1) may be written as

$$(\overline{\mathrm{HETP}})_o=2\lambda d_p+\frac{2\gamma D_{g,o}}{U_o}+\frac{16K\left(\frac{F_g}{F_l}\right)d_f^2}{\pi^2\left(1+K\frac{F_g}{F_l}\right)^2 D_l}\cdot\frac{U_o}{\left(1+\sqrt{1+\frac{360\eta_l U_o(1-\epsilon)^2}{P_o\epsilon^3 d_p^2}}\right)}\quad\ldots(3)$$

Consider now a column packed with a suitable non-volatile liquid distributed on spherical non-porous granules. If the percentage of liquid is held constant and the diameter of the granules is changed the effective thickness of the liquid film will change. By assuming that the liquid is uniformly distributed over the surface of the granules the actual thickness of the liquid layer may be calculated.

Let S =surface area per unit bed volume
S_o =specific surface area.

Then $S_o=\dfrac{6}{d_p}$ and
$S=S_o(1-\epsilon)$

The volume of liquid per unit bed volume, V_l, is given by

$$V_l=Sd_f=6d_f(1-\epsilon)/d_p$$

and similarly the volume of gas per unit bed volume is given by

$$V_g=\epsilon$$

Thus $V_g/V_l=F_g/F_l=\epsilon d_p/6d_f(1-\epsilon)$ .. .. (4)

The volume of the granules per unit bed volume, V_s, is

$$V_s=1-\epsilon \text{ (for thin liquid layers)}$$

The percentage of liquid phase in the column packing, P_l, is given by

$$P_l=\frac{\text{mass liquid per unit bed volume}\times 100}{\text{(mass liquid + mass granules) per unit bed volume}}$$
$$=100\,V_l\,\rho_l/(V_l\rho_l+V_s\rho_s)\quad\ldots\ldots(5)$$

where ρ_l =density of liquid
ρ_s =density of granules

If the percentage of liquid is low

$$P_l=100\,V_l\rho_l/V_s\rho_s$$
$$=100F_l\rho_l/F_s\rho_s$$
$$=600d_f\rho_l/d_p\,\rho_s\quad\ldots\ldots(6)$$

From equations (4) and (6) it follows that if the percentage of liquid in the column is held constant, the thickness of the liquid film is proportional to the diameter of the non-porous spheres and also that F_g/F_l is constant.

From equations (3), (4) and (6) it follows that
$(\overline{\mathrm{HETP}})_o=$

$$2\lambda d_p+\frac{2\gamma D_{g,o}}{U_o}+\frac{K\left(\frac{\epsilon}{1-\epsilon}\right)\left(\frac{P_l d_p^2\rho_s}{\rho_l}\right)}{225\pi^2\left(1+\frac{100\epsilon\rho_l K}{(1-\epsilon)\rho_l\rho_s}\right)^2 D_l}\cdot\frac{U_o}{\left(1+\sqrt{1+\frac{360\eta_l U_o(1-\epsilon)^2}{P_o\,\epsilon^3 d_p^2}}\right)}\quad\ldots(7)$$

If all the column parameters, other than the particle diameter, the percentage of liquid phase, and the linear gas velocity at the outlet, are held constant, equation (7) reduces to

$$(\overline{\mathrm{HETP}})_o = 1{\cdot}2d_p + \frac{B}{U_o} + \frac{C.P_l d_p^2}{(1+D/P_l)^2} \cdot \frac{U_o}{\left(1+\sqrt{1+\frac{EU_o}{d_p^2}}\right)} \quad \cdots \cdots \cdots \cdots \quad (8)$$

where[2] $2\lambda = 1{\cdot}2$
and $\gamma = 0{\cdot}667$
$B = 1{\cdot}33\ D_{g,o}$
$C = K\epsilon\rho_s/225\pi^2(1-\epsilon)D_l\rho_l$
$D = 100K\epsilon\rho_l/(1-\epsilon)\rho_s$
$E = 360\eta l(1-\epsilon)^2/P_o\epsilon^3$

Although the following values, which have been chosen for the various parameters, are hypothetical they might well occur in practice.

$D_l = 5\times10^{-7}\,\mathrm{cm^2\,sec^{-1}}$
$D_{g,o} = 0{\cdot}075\,\mathrm{cm^2\,sec^{-1}}$
$\eta = 2\times10^{-4}$ poise
$P_o = 10^6\,\mathrm{dynes\,cm^{-2}}$
$l = 100$ cm
$\rho_s/\rho_l = 1{\cdot}5$
$\epsilon = 0{\cdot}4$
$d_p = 0{\cdot}05, 0{\cdot}03, 0{\cdot}025, 0{\cdot}015$ cm
$P_l = 2, 4, 8\%$
$K = 0{\cdot}009$
$B = 0{\cdot}1\,\mathrm{cm^2\,sec^{-1}}$
$C = 8{\cdot}108$
$D = 0{\cdot}4004$
$E = 4{\cdot}05\times10^{-5}$ cm sec.

Equation (8), and values of the various parameters chosen from the above list, have been used to calculate the mean height equivalent per theoretical plate for various values of the linear gas velocity at the column outlet. The results are shown in Fig. 1. The minimum mean height equivalent per theoretical plate, for each value of P_l and d_p has been obtained from these curves and has been plotted against the particle diameter (Fig. 2) and against the percentage of liquid phase (Fig. 3).

DISCUSSION

From the results in Figs. 1-3 the following conclusions may be drawn. For each percentage of liquid phase and for each particle diameter there is an optimum value of the gas velocity at the outlet for which the mean height equivalent per theoretical plate is a minimum. The optimum gas velocity at the outlet increases as either the particle diameter and/or the percentage of liquid is decreased. For relatively large granules and/or large percentages of liquid the optimum gas velocity at the outlet is fairly critical which implies that a deviation of the gas velocity at the outlet from the optimum value can cause a relatively large decrease in the column efficiency. Where the percentage of liquid phase and/or the particle diameter is small the linear

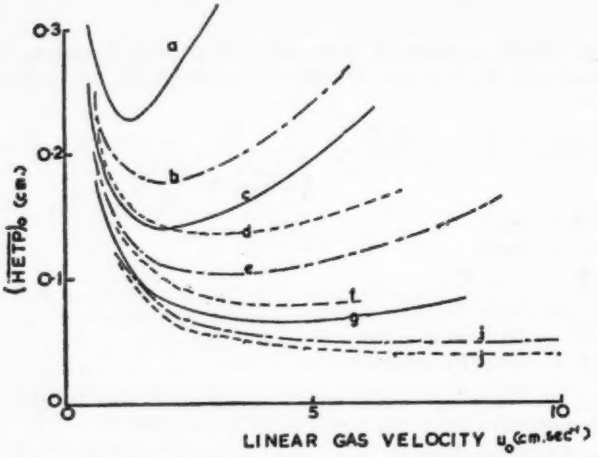


FIG. 1.—The influence of the particle diameter and of the percentage of liquid phase on the variation of the $(\overline{\mathrm{HETP}})_o$ with increasing linear outlet gas velocity

a: Calculated from equation (8) with $P_l=8\%$ and $d_p=0{\cdot}05$ cm
b: Calculated from equation (8) with $P_l=4\%$ and $d_p=0.05$ cm
c: Calculated from equation (8) with $P_l=8\%$ and $d_p=0{\cdot}03$ cm
d: Calculated from equation (8) with $P_l=2\%$ and $d_p=0{\cdot}05$ cm
e: Calculated from equation (8) with $P_l=4\%$ and $d_p=0{\cdot}03$ cm
f: Calculated from equation (8) with $P_l=2\%$ and $d_p=0{\cdot}03$ cm
g: Calculated from equation (8) with $P_l=8\%$ and $d_p=0{\cdot}015$ cm
i: Calculated from equation (8) with $P_l=4\%$ and $d_p=0{\cdot}015$ cm
j: Calculated from equation (8) with $P_l=2\%$ and $d_p=0{\cdot}015$ cm

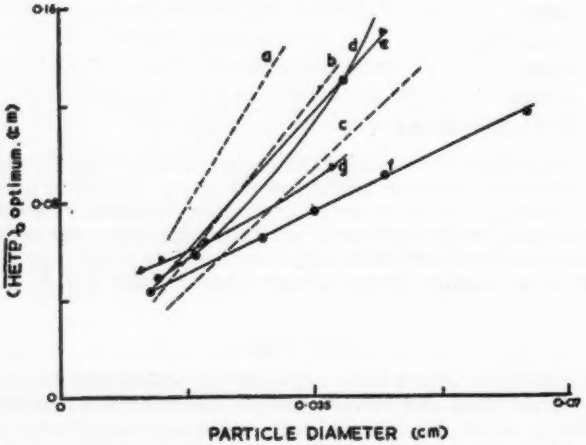


FIG. 2.—The effect of the particle diameter on the optimum column efficiency

a: Theoretically calculated from equation (8) for glass with $P_l=8\%$
b: Theoretically calculated from equation (8) for glass with $P_l=4\%$
c: Theoretically calculated from equation (8) for glass with $P_l=2\%$
d: Results obtained by Desty *et al*[9] for Celite with $P_l=10\%$
e: Results obtained by Desty *et al*[9] for Celite with $P_l=20\%$
f: Results obtained by Bohemen & Purnell[8] for Silocel with $P_l=20\%$
g: Results obtained by Cheshire & Scott[10] for Sterchamol fire-brick with $P_l=20\%$

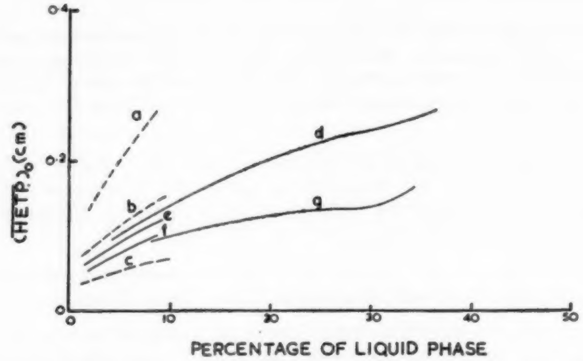


FIG. 3.—The effect of the percentage of liquid phase on the optimum column efficiency

a: Theoretically calculated from equation (8) for glass spheres with $d_p=0.05$cm
b: Theoretically calculated from equation (8) for glass spheres with $d_p=0{\cdot}03$ cm
c: Theoretically calculated from equation (8) for glass spheres with $d_p=0{\cdot}025$ cm
e: Theoretically calculated from equation (8) for glass spheres with $d_p=0{\cdot}015$ cm
d: Results obtained by Keulemans, *et al.*[4] for Sterchamol firebrick with $d_p=0{\cdot}044$ cm
f: Results obtained by de Wet & Pretorius[5] for Sterchamol firebrick with $d_p=0{\cdot}01$ cm
g: Results obtained by de Wet & Pretorius[5] for Celite for $d_p=0{\cdot}01$ cm

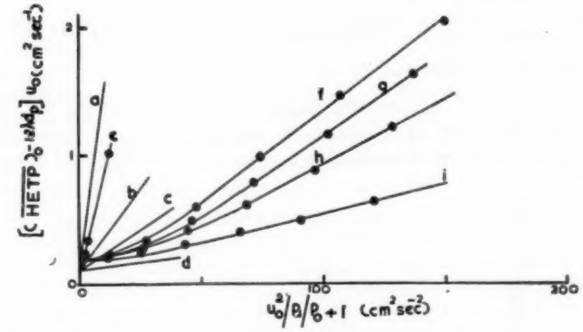


FIG. 4.—$((\overline{HETP})_o - 1{\cdot}2\lambda d_p)\,U_o$ plotted against $U_o^2/\,P_i/P_o + 1$

a: Theoretically calculated from equation (8) for glass spheres with $P_l=8\%$ and $d_p=0{\cdot}05$ cm
b: Theoretically calculated from equation (8) for glass spheres with $P_l=2\%$ and $d_p=0{\cdot}05$ cm
c: Theoretically calculated from equation (8) for glass spheres with $P_l=8\%$ and $d_p=0{\cdot}015$ cm
d: Theoretically calculated from equation (8) for glass spheres with $P_l=2\%$ and $d_p=0{\cdot}015$ cm
e: Results obtained by Littlewood[7] for glass beads with $P_l=1\%$ and $d_p=0{\cdot}01$ cm
f: Results obtained by Bohemen & Purnell[8] for Silocel with $P_l=20\%$ and $d_p=0{\cdot}064$ cm
g: Results obtained by Bohemen & Purnell[8] for Silocel with $P_l=20\%$ and $d_p=0{\cdot}0445$ cm
h: Results obtained by Bohemen & Purnell[8] for Silocel with $P_l=20\%$ and $d_p=0{\cdot}035$ cm
i: Results obtained by Bohemen & Purnell[8] for Silocel with $P_l=20\%$ and $d_p=0{\cdot}028$ cm

gas velocity at the outlet may be substantially increased beyond the optimum value without significantly impairing the column efficiency. In analytical work this fact is important since it means that the time required to elute the components of a mixture from the column may be shortened without detracting from the efficiency of the separation.

If the column is operated at the optimum outlet gas velocity the mean height equivalent per theoretical plate decreases as either the percentage of liquid or the particle diameter is decreased. In both cases the increase in efficiency may be ascribed to a decrease in the thickness of the liquid film.

The above conclusions are strictly valid for non-porous spheres only; at present sufficient experimental data with which to test them are still lacking.

The experimental results which have been obtained using porous granules are, however, in general agreement with the theoretically predicted behaviour of non-porous spheres, as is shown in Figs. 2 and 3. One noticeable exception is apparent. The mean height equivalent per theoretical plate of a column packed with porous granules tends to become independent of the particle diameter for very small particles if the percentage of liquid phase is held constant. This would suggest that the "effective" thickness of the liquid film on porous granules does not decrease beyond a certain limit. Further evidence in support of this conclusion may be obtained as follows:

Equation (8) may be written as

$$[(\overline{\mathrm{HETP}})_o - 1{\cdot}2d_p]U_o = B + C'\,U_o^2/(1+P_i/P_o) \qquad \ldots\ldots\ldots\ldots \qquad (9)$$

where $C' = C\,P_l d_p^2/(1+D/P_l)^2$

It is assumed that all the column parameters other than d_p, U_o, P_i and P_o are constant. The left-hand side of equation (9) has been plotted against $U_o^2/(1+P_i/P_o)$ for various values of the particle diameter (Fig. 4). The values of the various para-

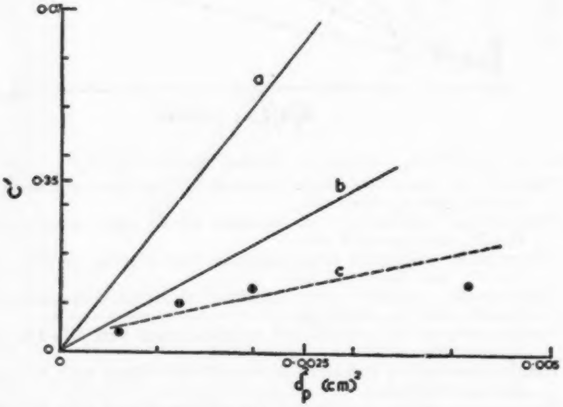


FIG. 5.—C′ plotted against (d_p^2)

a Theoretically calculated from equation (8) for glass spheres with P_l=4%
b Theoretically calculated from equation (8) for glass spheres with P_l=2%
c Obtained from the results of Bohemen & Purnell[8] in Fig. 4 for Silocel with P_l=20%

meters have been chosen from the list given above. For each set of parameters the corresponding value of the inlet pressure has been calculated from equation (2). Similar curves have been obtained from the experimental data of Littlewood, *et al.*[7] for glass spheres, and of Bohemen and Purnell[8] for porous granules (Silocel) and are also shown in Fig. 4. Since these authors have not stated the pressures at which their experiments were carried out it has been necessary to assume $P_o=1$ atm and to calculate P_i from equation (2). Good agreement between the experimental results and the theory is obtained at high but not at low gas velocities. This discrepancy suggests that the van Deemter equation should include an extra term to account for transverse diffusion effects analogous to that incorporated in the equation derived by Golay for capillary columns[6].

According to equation (9) the slope of each curve in Fig. 4 gives the corresponding value of C'. Because of the difficulties previously mentioned the slopes should be measured at high outlet gas velocities in the case of the experimental curves. Each slope has been plotted against the corresponding value of (d_p^2) in Fig. 5.

If, at constant percentage of liquid, the film thickness is directly proportional to the particle diameter, the curve in Fig. 5 will be a straight line with a pronounced slope. For porous granules this does not appear to be true; the effective thickness of the liquid film is virtually independent of the particle diameter. This result may be partially explained by the fact that in contrast to non-porous granules, the specific surface area of porous granules does not change significantly as the particle diameter is decreased[4].

It is reasonable to assume that the highest column efficiencies will be achieved by using non-porous granules of very small diameter on which the minimum amount of liquid necessary to cover the surface is spread. In the extreme case where the liquid phase forms a mono-molecular layer, the distinction between partition and adsorption processes will disappear. A detailed experimental study of gas-chromatography processes under such conditions should do much to facilitate the development of a general theory covering both gas-solid and gas-liquid chromatography. Work along these lines is at present proceeding in these Laboratories.

Because of the preferential inclusion by capillary action of the liquid in the finer pores of a porous granule, it is unlikely that very thin uniform liquid films will be achieved. The effective thickness of the liquid layer on such granules will be an average of the actual thickness of the film at all points on the surface of the granule. If very small amounts of liquid are deposited on porous granules, wells of liquid in the finer pores and bare patches in the more open surfaces appear to result.[4] The inherently high efficiency of a column containing non-porous granules on which a very thin liquid film has been distributed will only be realised if the sample volume is correspondingly small.[5]

The senior author (V.P.) is indebted to African Explosives & Chemical Industries, Ltd., for the award of a Research Fellowship and to the Council for Scientific and Industrial Research for financial assistance.

Department of Physical Chemistry,
University of Pretoria.

†Central Tobacco Research Station.
Kroondal,
Transvaal.

*Department of Physics,
University of Potchefstroom.

Received October 27, 1959.

REFERENCES

[1] van Deemter, Zuiderweg and Klingkenberg, *Chem. Eng. Sci.*, **5**, 271, 1956.
[2] de Wet and Pretorius, *preceding paper*.
[3] Carman, *Discussions Faraday Soc.*, No. 3, 72, 1948.
[4] Keulemans, *Gas Chromatography*, Rheinhold Publishing Corporation, New York, 1957, p. 144.
[5] de Wet and Pretorius, *in the press*.
[6] Golay, *Gas Chromatography*, edited by Desty, Butterworths Scientific Publications, London, 1958, p. 36.
[7] Littlewood, *ibid.*, p. 23.
[8] Bohemen and Purnell, *ibid.*, p. 6.
[9] Desty, Godfrey and Harbourn, *ibid.*, p. 200.
[10] Cheshire and Scott, *J. Inst. Petroleum*, **44**, 74, 1958.

cis-trans ISOMERISM OF AMINOAZO COMPOUNDS*

by

J. DIJKSTRA

OPSOMMING

'n Asoverbinding kan voorkom as 'n stabiele *trans*-isomeer of 'n onstabiele *cis*-isomeer. Laasgenoemde besit 'n hoër elektronedigtheid op die asogroep. Die stabiliteite van die *cis*-isomere van aminoasoverbindings is ondersoek in alkaliese etielalkohol-watermengsels en die snelheid van *cis-trans*-isomerisasie is gemeet. Die halfleeftyd van die *cis*-isomere verminder met afnemende alkoholkonsentrasie en met afnemende pH. Die halfleeftyd van die *cis*-isomere van 4-(dialkielamino)-asobenseenderivate word verminder deur N-dealkilering en deur 2'-karboksilering (metielrooi) en 4'-sulfonering (metieloranje); dit word aansienlik verhoog deur 2'-metielsubstitusie. Die resultate word bespreek in die lig van 'n moontlike verband met die kankerverwekkende aktiwiteit van aminoasoverbindings.

SUMMARY

An azo compound can exist as a stable *trans* isomer or an unstable *cis* isomer. The latter has a higher electron density at the azo group. The stabilities of the *cis* isomers of aminoazo compounds have been studied in alkaline ethyl alcohol-water mixtures and the rate of *cis-trans* isomerization has been measured. The half-life of the *cis* isomers decreases with decreasing alcohol concentration and with decreasing pH. The half-life of the *cis* isomers of 4-(dialkylamino)-azobenzene derivatives is decreased by N-dealkylation and by 2'-carboxylation (methyl red) and 4'-sulphonation (methyl orange); it is significantly increased by 2'-methyl substitution. These results are discussed in the light of a possible relation to the carcinogenic activity of aminoazo compounds.

INTRODUCTION

The existence of two isomeric forms of aromatic azo compounds was discovered by Hartley in 1937[1]. The *trans* isomer can assume a coplanar structure which is stabilised by resonance. In contract, interference of the *ortho* hydrogen atoms prevents coplanarity in the *cis* isomer[2]. Consequently, the *trans* isomer is more stable than the *cis* isomer. In a few cases, crystalline *cis* azo compounds have been prepared which appear to be quite stable in the dark at room temperature.[3,4] In most cases, however, the *cis* isomer has been studied in solutions in organic solvents where it is formed by partial conversion of the *trans* isomer on exposure to light.[3] On standing in the dark it reverts completely to the *trans* isomer.

In the aminoazobenzene series the *cis-trans* isomerism of only 4-aminoazobenzene and 4-dimethylaminoazobenzene has been studied, both in organic solvents[3,5,6,7]. Whereas the *trans* isomers could be partly converted into the *cis* form by illumination in solvents such as isooctane, methylethylketone, benzene, pyridine and dimethylformamide, no *cis* isomer formation was observed in alcoholic and aqueous solutions. It was, however, observed by Thiel and Springemann[8] in 1928 that dimethylaminoazobenzene, methyl red and methyl orange were light-sensitive in alkaline alcohol-water mixtures. Although these authors suggested that the dyes are activated by light, and in the activated state combine with the alcohol with the disappearance of the azo chromophore, the results may also be taken as an indication of the formation of *cis* isomers. It was therefore considered to be of interest to extend the study of *cis-trans* isomerization of aminoazo compounds to alkaline alcohol-water mixtures. In all, ten compounds were studied, including several well-known carcinogens.

---

*A paper read in Pretoria at the Thirteenth Annual Convention of the S.A. Chemical Institute on July 24, 1959.

EXPERIMENTAL

Reagents. The following dyes were prepared by coupling diazotized aromatic amines with the appropriate amines as described by Miller and Miller[9]: 4-aminoazobenzene (AB; m.p. 124·0—125·0°), 4-monomethylaminoazobenzene (MAB; m.p. 88·6—89·0°), 4-dimethylaminoazobenzene (DAB; m.p. 118·0—119·0°), 2′-methyl-4-dimethylaminoazobenzene (2′-me-DAB; m.p. 74·0—75·0°), 3′-methyl-4-dimethylaminoazobenzene (3′-me-DAB; m.p. 120·4—121·2°), 4′-methyl-4-dimethylaminoazobenzene (4′-me-DAB; m.p. 172·1—172·9°), 4-monoethylaminoazobenzene (EAB; m.p. 87·7—88·3°), 4-diethylaminoazobenzene (DEAB; m.p. 97·4—98·4°). AnalaR methyl orange (MO) (obtained from Hopkin and Williams) and AnalaR methyl red (MR) (obtained from BDH) were also used.

Cis-trans isomerization was studied in alkaline ethyl alcohol-water mixtures containing sodium hydroxide or borate buffer. The stock solution of 0·2M borate buffer contained 12·37 g H_3BO_3 and 4·27 g sodium hydroxide per litre. The dye concentration was usually 10^{-5}M. When the alcohol concentration was less than 39% v/v, the dye concentration was reduced to 10^{-6}M.

Formation of cis isomer. For the partial conversion of stable *trans* isomers into the *cis* forms, solutions of aminoazo compounds were illuminated at constant temperature with a 140 watt Hanovia mercury vapour lamp at a distance of 30 cm for 10 min. (Glass plates were placed between the lamp and the solutions to eliminate ultraviolet light.)

Measurement of cis-trans isomerization. Optical densities were measured on a Beckmann spectrophotometer, Model DU, at controlled temperatures using a 10 mm silica absorption cell for the 10^{-5}M dye solutions and a 10 cm silica absorption cell for the 10^{-6}M solutions. The quantitative estimation of *cis* isomer is usually based on the fact that the absorption spectra of the *cis* isomers differ considerably from those of the *trans* isomers[4, 5, 10]. Consequently, formation of *cis* isomer by illumination leads to a drop in the absorption band of the *trans* isomer and the concentration of *cis* isomer at any time is proportional to this drop. The conversion of the *cis* isomer into the *trans* isomer in the dark is accompanied by an increase in the optical density at the absorption maximum of the *trans* isomer. This conversion was readily followed on the spectrophotometer. The intensity of the measuring beam was found to be too low to cause any isomerization. The results are expressed by means of the first-order rate constant (k) of the *cis*→*trans* isomerization and the period of half-life ($t\frac{1}{2}$) of the *cis* isomer.

RESULTS

The dark reactions of aminoazo compounds in alkaline ethyl alcohol-water mixtures followed without exception a first-order rate law. Typical results for the change in optical absorption in the case of DAB on illumination (light effect) and subsequent standing in the dark (dark reaction) are shown in Table I and Figs. 1 and 2. From the rate constants of the dark reaction at 13·9° and 33·3°C the energy of activation, calculated by the Arrhenius equation, was found to be 20·0 kcal/mole. A value of $10^{11\cdot 1}$ sec^{-1} was calculated for the collision constant A in the Arrhenius equation $k = Ae^{-E/RT}$.

TABLE I

Rate constants of cis→trans *isomerization of DAB in O·02N NaOH solution containing* 39% *v/v ethyl alcohol. Optical density measured at* 430 *mμ*

Temperature	Light effect: decrease of optical density (%)	Dark reaction	
		k (sec^{-1})	$t_{\frac{1}{2}}$ of cis isomer (sec)
13·9°	42	$7 \cdot 44 \times 10^{-5}$	9310
33·3°	41	$6 \cdot 80 \times 10^{-4}$	1020

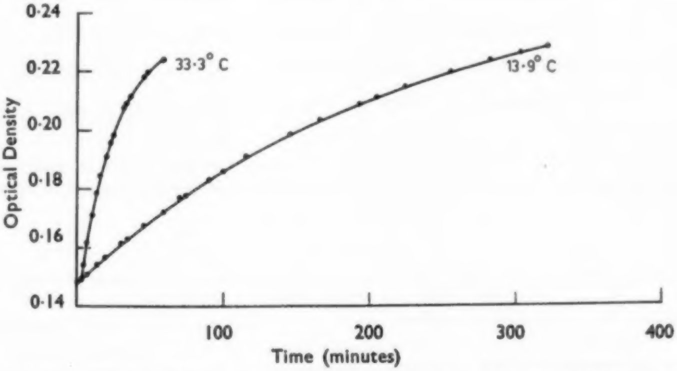


FIG. 1.—Change of optical density during the dark reaction of DAB at 13·9°C and 33·3°C

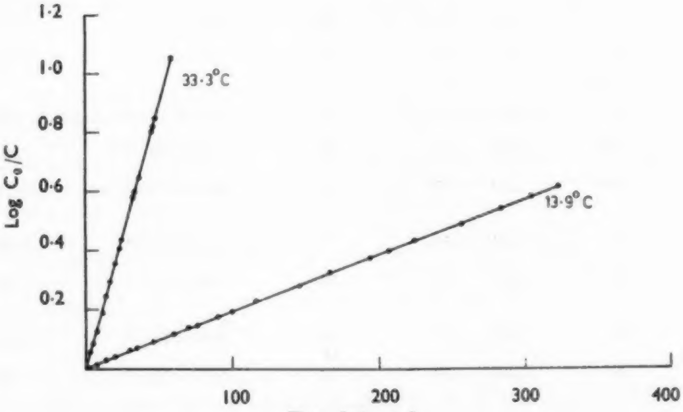


FIG. 2.—Rate of *cis-trans* isomerization of DAB at 13·9°C and 33·3°C (C is concentration of *cis* isomer. C_0 is initial concentration)

The effect of pH on the isomerism of DAB and methyl orange is shown in Table II. The rate of *cis* to *trans* isomerization increases considerably with decreasing pH.

TABLE II

Effect of pH on the cis-trans *isomerization of DAB and methyl orange. Optical density measured at* 430 *mμ*

Dye	EtOH (% v/v)	Buffer	pH	Temp. °C	Light effect: decrease of optical density (%)	Dark reaction	
						k (sec^{-1})	$t_{\frac{1}{2}}$ of *cis* isomer (sec)
DAB	39	0·02N NaOH	12·6	33·3	41	$6{\cdot}80 \times 10^{-4}$	1020
DAB	39	0·10M borate	10·5	34·5	7	$6{\cdot}0 \times 10^{-3}$	115
MO	76	0·02N NaOH	12·6	29	41	$3{\cdot}80 \times 10^{-4}$	1820
MO	76	0·02M borate	11·6	29	33	$1{\cdot}6 \times 10^{-3}$	436

From Table III it is clear that the *cis* to *trans* isomerization is accelerated by decreasing the alcohol concentration, and hence by increasing amounts of water.

TABLE III

Effect of solvent on the cis-trans *isomerization of dimethylaminoazo compounds in* 0·02N NaOH *solutions containing varying amounts of ethyl alcohol*

Dye	EtOH in solvent (% v/v)	pH	Temp. (°C)	Light effect: decrease of optical density (%)	Dark reaction	
					k (sec^{-1})	$t_{\frac{1}{2}}$ of *cis* isomer (sec)
DAB	76	12·6	29	55	$9{\cdot}40 \times 10^{-5}$	7370
DAB	39	12·6	29	42	$4{\cdot}30 \times 10^{-4}$	1610
DAB	19	12·5	21	30	$1{\cdot}61 \times 10^{-3}$	431
DAB	9·5	12·4	21	26	$4{\cdot}58 \times 10^{-3}$	151
DAB	4·8	12·3	21	21	$8{\cdot}82 \times 10^{-3}$	79
MO	76	12·6	29	41	$3{\cdot}80 \times 10^{-4}$	1820
MO	39	12·6	29	16	$7{\cdot}68 \times 10^{-3}$	90
MR	76	12·6	29	40	$3{\cdot}63 \times 10^{-4}$	1910
MR	39	12·6	29	40	$8{\cdot}03 \times 10^{-3}$	86

Observations on the *cis-trans* isomerization of various aminoazo compounds under standard conditions are summarised in Table IV.

TABLE IV

cis-trans *Isomerization of various aminoazo compounds in* 0·02N NaOH *solutions containing* 39% *v/v ethyl alcohol and the relative carcinogenic activity of these compounds*

Dye	Temp. (°C)	Wavelength of experiment (mμ)	Light effect: decrease of optical density (%)	Dark reaction		Relative carcinogenic activity
				k (sec^{-1})	$t_{\frac{1}{2}}$ of *cis* isomer (sec)	
DAB	33·3	430	41	$6·80 \times 10^{-4}$	1020	6
2′-me-DAB	33·3	420	40	$1·60 \times 10^{-4}$	4320	2—3
3′-me-DAB	32·6	430	42	$5·72 \times 10^{-4}$	1210	12
4′-me-DAB	33·3	420	50	$6·28 \times 10^{-4}$	1100	<1
4′-SO_3Na-DAB(MO)	33·6	456	20	$8·4 \times 10^{-3}$	83	0
2′-COONa-DAB(MR)	33·6	420	19	$1·6 \times 10^{-3}$	43	0
DEAB	33·0	450	38	$9·81 \times 10^{-4}$	706	0
MAB	21 to 34	406,460	0	—	—	6
EAB	21 to 34	410,460	0	—	—	0
AB	21 to 34	382,440	0	—	—	0

As compared to DAB, the *cis* isomer of 2′-methyl-DAB has a significantly larger $t_{\frac{1}{2}}$, while the *cis* isomers of the 3′- and 4′-methyl derivatives have only slightly larger $t_{\frac{1}{2}}$ values. The 4′-sulphonate group of MO and the 2′-carboxylate group of MR decrease the $t_{\frac{1}{2}}$ of the *cis* isomer. The *cis* isomer of DEAB is also less stable than *cis*-DAB. No *cis* isomer formation could be detected in alkaline ethyl alcohol-water (39% v/v alcohol) solutions of aminoazobenzene (AB) and monomethyl- and monoethyl-AB. In the case of AB, increasing the alcohol concentration to 76% still had no effect.

Using the same light source, *cis* isomer formation of these compounds does occur in methylethylketone. For example, in the case of AB, illumination caused a 65% decrease in the optical density of the *trans* isomer and the subsequent dark reaction had a half-life of 565 seconds. The wavelengths of maximum absorption of these three compounds in the alkaline alcohol-water mixtures used differed only slightly from those in methylethylketone. Since it has been reported that light absorbed in the wavelength range of the main absorption band of the *trans* isomer is most effective in bringing about *trans* into *cis* conversion[5,11], it follows that the failure to detect *trans*→*cis* isomerization of AB, MAB and EAB in alkaline alcohol-water mixtures is not due to illumination by light of an unsuitable wavelength, but to the high rate of the reverse reaction.

As aminoazo compounds are bound by serum and liver proteins[12,13], it was felt worth while to investigate the effect of protein on the *cis-trans* isomerism of DAB. However, no *cis* isomer formation could be detected in 10^{-6}M DAB solution in 0·1M borate buffer of pH 9·2, either in the presence of 0·2% bovine serum albumin or in the presence of diluted rat serum and 0·15M sodium chloride.

DISCUSSION

The reversible light effect on the optical absorption of aminoazo compounds in alkaline ethyl alcohol-water mixtures has all the characteristics of *cis-trans* isomerism. A further indication that the changes are due to *cis-trans* isomerism is the fact that the observed energy of activation of 20·0 kcal/mole in the case of DAB is of the same order as the values 19-23 kcal per mole found for the *cis-trans* isomerization of various azo compounds in organic solvents[3, 7, 11, 14, 15]. The magnitude of the collision constant A suggests that under the experimental conditions the isomerization is a normal thermal rearrangement[15].

The considerable increase of the rate of *cis* to *trans* conversion with decreasing pH (Table II) is analogous to the observation in organic solvents that the *cis*→*trans* isomerization of aminoazo compounds is accelerated by traces of acids[3] and solvents with proton-donor ability.[7] Hartley[3] suggested that protonation of the azo group facilitates the isomerization.

The observed accelerating effect of water on the dark *cis*→*trans* isomerization (Table III) is only partly due to a pH effect, since the accompanying decrease of pH is small. It is therefore attributed to solvation, the extent of which increases with increasing polarity of the solvent when replacing alcohol by water. Brode[16] has suggested that the stable *trans* isomers of aminoazo compounds add water and even alcohols to the azo group by means of hydrogen bonding. Since *cis* isomers have a greater electron density at the azo group[17], a similar solvation of *cis* isomers is possible. At the high pH values used in the present investigation, this solvation may take the place of protonation in the reaction mechanism of Hartley.

In 2′-methyl-DAB a primary steric effect of the *ortho* methyl group and a weak hydrogen bond between the methyl group and the azo group[18], hindering solvation of the azo group, may be the cause of the decrease of the rate of *cis* to *trans* isomerization.

Since various 4-aminoazobenzene derivatives induce liver tumours[19], it is of interest to compare the carcinogenic activity of the compounds studied with the relative stability of their *cis* isomers. Pullman has suggested that carcinogenic activity is associated with a high electron density at the azo linkage[20, 21]. In this connection it is of interest that *cis*-azo compounds have a greater electron density at the azo linkage[17] than the *trans* isomers, and are more reactive with respect to oxidation[17] and reduction[22]. From Table IV it appears that in alkaline alcohol-water mixtures the half-lives of the *cis* isomers of most of the carcinogenic aminoazobenzenes studied are of the same order and are significantly greater than those of related non-carcinogenic derivatives. There are, however, two noteworthy exceptions: DEAB, which is non-carcinogenic, has a *cis* isomer with a half-life of the same order as those of the *cis* isomers of the carcinogens DAB, 3′-me-DAB and 4′-me-DAB; on the other hand, the *cis* isomer of MAB is very unstable, although MAB is a potent carcinogen. The present work has also shown that no appreciable *cis* isomer formation can be expected under physiological conditions, *i.e.*, in the absence of alcohol and at pH values in the region of 7. Hence, the relationship between *cis-trans* isomerization and carcinogenicity, if any, cannot be a simple one.

To Dr. H. M. Schwartz and Dr. F. J. Joubert I am greatly indebted for interest and criticism at all times.

This project was sponsored by the National Cancer Association of South Africa, and the paper is published with the permission of the South African Council for Scientific and Industrial Research.

National Chemical Research Laboratory,
South African Council for Scientific and Industrial Research,
Pretoria.

Received October 22, 1959.

REFERENCES

[1] Hartley, *Nature*, 1937, **140**, 281.
[2] Robertson, *J. Chem. Soc.*, **1939**, 232.
[3] Hartley, *ibid.*, **1938**, 633.
[4] Birnbaum, Linford and Style, *Trans. Faraday Soc.*, 1953, **49**, 735.
[5] Brode, Gould and Wyman, *J. Am. Chem. Soc.*, 1952, **74**, 4641.
[6] Brode, Gould and Wyman, *ibid.*, 1953, **75**, 1856.
[7] Horowitz and Klotz, *ibid.*, 1955, **77**, 5011.
[8] Thiel and Springemann, *Z. anorg. u. allgem. Chem.*, 1928, **176**, 112.
[9] Miller and Miller, *J. Exptl. Med.*, 1948, **87**, 139.
[10] Cook, Jones and Polya, *J. Chem. Soc.*, **1939**, 1315.
[11] Fischer and Frei, *J. Chem. Phys.*, 1957, **27**, 328.
[12] Kubo, Kobayashi and Wada, *Med. J. Osaka Univ.*, 1955, **6**, 575.
[13] Burkhard, Bauer and Grossman, *J. Am. Chem. Soc.*, 1957, **79**, 3209.
[14] Atherton and Peters, *Spec. Publ. Chem. Soc.* (London), **1956** (No. 4), 17.
[15] Schulte-Frohlinde, *Ann.*, 1958, **612**, 138.
[16] Brode, Seldin, Spoerri and Wyman, *J. Am. Chem. Soc.*, 1955, **77**, 2762.
[17] Badger, Buttery and Lewis, *J. Chem. Soc.*, **1953, 2143**.
[18] Buraway and Thompson, *ibid.*, **1953**, 1443.
[19] Miller and Miller, *Adv. Cancer Research*, 1953, **1**, 339.
[20] Pullman, *Compt. rend.*, 1946, **222**, 1501.
[21] Pullman and Pullman, *Rev. Sci.*, 1946, **84**, 145.
[22] Winkel and Siebert, *Ber.*, 1941, **74B**, 670.

RADIOLYSIS OF FERROUS SULPHATE SOLUTIONS WITH STANDARDISED INTERNAL SOURCES OF PHOSPHORUS-32*

by

M. PEISACH† and J. STEYN‡

OPSOMMING

Die radiolitiese opbrengs van ferri-ione in die "Fricke"-dosismeteroplossing (10^{-3}M ferroammoniumsulfaat en 10^{-3}M natriumkloried in lugversadigde 0·8N swawelsuur) is bepaal deur middel van interne bestraling met fosfor-32. Die radioaktiwiteit is gestandaardiseer met beide 4π-eweredigheidstelling en 4π-vloeistofsintillasietelling. Dit is bekend dat hierdie twee telmetodes vir fosfor-32 resultate lewer wat 1% van mekaar verskil en gevolglik is respektieflike G-waardes van 10·62 $\overline{E}^{-1}$ en 10.50 $\overline{E}^{-1}$ ione per 100 eV gevind, waar $\overline{E}$ die gemiddelde energie is wat per disintegrasie vrygestel word deur fosfor-32. Met $\overline{E}=0{\cdot}690$ MeV is hierdie G-waardes ooreenstemmend 15·39 ± 0·04 en 15·21 ± 0·04.

SUMMARY

The radiolytic yield of ferric ions in the "Fricke" dosimeter solution (10^{-3}M ferrous ammonium sulphate and 10^{-3}M sodium chloride in air-saturated 0·8N sulphuric acid) was determined with internal sources of phosphorous-32. The radioactivity was standardised with 4π-proportional counting and 4π-liquid scintillation counting methods. These two procedures which are known to give results differing by about 1% for phosphorous-32 gave G-values of 10·62 $\overline{E}^{-1}$ and 10·50 $\overline{E}^{-1}$ ions per 100 eV respectively, where $\overline{E}$ is the average energy dissipated per disintegration of phosphorus-32. For $\overline{E}=0{\cdot}690$ MeV the G-values are correspondingly 15·39 ± 0·04 and 15·21 ± 0·04.

INTRODUCTION

The simplest and most widespread chemical dosimeter for the measurement of radiation dosage is the "Fricke" dosimeter which consists of a 10^{-3}M solution of ferrous sulphate in air-saturated 0·8N sulphuric acid.[1] The initial effect of ionising radiation on an aqueous system is to produce hydrogen atoms, hydroxyl radicals, molecular hydrogen peroxide and molecular hydrogen and possibly other chemical species in much lower concentrations. These primary products may now oxidise the dissolved ferrous ions by the reactions:

$$\begin{array}{rcl}
Fe^{++} + OH & \longrightarrow & Fe^{+++} + OH^{-} \\
H + O_2 & \longrightarrow & HO_2 \\
HO_2 + Fe^{++} & \longrightarrow & Fe^{+++} + HO_2^{-} \\
HO_2^{-} + H^{+} & \rightleftarrows & H_2O_2 \\
H_2O_2 + Fe^{++} & \longrightarrow & Fe^{+++} + OH + OH^{-}
\end{array}$$

The effect of organic impurities (RH) in the system is to increase the ferric ion concentration by the chain involving the reactions:

$$\begin{array}{rcll}
OH + RH & \longrightarrow & R\cdot & + H_2O \\
R\cdot + O_2 & \longrightarrow & RO_2\cdot & \\
RO_2\cdot + Fe^{++} & \longrightarrow & RO_2^{-} & + Fe^{+++} \\
RO_2^{-} + H^{+} & \longrightarrow & ROOH & \\
ROOH + Fe^{++} & \longrightarrow & RO^{-} & + Fe^{+++} + OH
\end{array}$$

---

*A paper read in Cape Town at the Fourteenth Annual Convention of the S.A. Chemical Institute on February 12, 1960.

but this chain reaction can be prevented by the addition of chloride which causes the oxidation of only one ferrous ion per hydroxyl radical by the reations:

$$\begin{aligned} OH + Cl^- &\longrightarrow OH^- + Cl \\ Fe^{++} + Cl &\longrightarrow Fe^{+++} + Cl^- \end{aligned}$$

Accordingly the "Fricke" dosimeter solution includes 10^{-3}M sodium chloride.

The determination of the G-value, *i.e.*, the yield in ions per 100 eV energy absorbed, of the dosimeter solution depends on the measurement of the increase in the ferric ion concentration and on a knowledge of the amount of energy absorbed by the system. To determine the ferric ion concentration spectrophotometric measurements are made of the optical density of the solution at 304-305 mμ, the absorption peak of ferric ions. To determine the energy, methods that have been used are ion current measurements, ionisation chamber methods, internal radiation methods[1] and colorimetry. In the early determinations there was a good deal of uncertainty about the G-value, results ranging from about 14 to 21[2,3], but more recently, in the period 1955 to 1957 the accepted value fell to between 15 and 17[4,5]. At present the most favoured value seems to be 15·5 but even this is not universally accepted.[6]

In the present publication the energy absorbed by the solution was determined from an accurate standardisation of the amount of radioactive material present in the solution, when carrier-free phosphorus-32 was dissolved in the dosimeter solution to act as radiation source.

EXPERIMENTAL

Principle of the method. To measure the radiation yield we define G as the number of ferric ions produced per 100 eV. Hence the number of ions produced by E MeV is

$$GE \times 10^4$$

and if E is the energy absorbed per c.c. of solution, then the concentration of ferric ions, c, in g ions per liter is

$$c = \frac{GE}{N} \times 10^7$$

where N is the Avogadro number.

For solutions which obey the Beer-Lambert law, we have

$$kc = \log \frac{I_0}{I_t} = D_t$$

where k is the molar extinction coefficient of the ferric solutions, D_t the optical density and I_t the intensity of transmitted light for the irradiated solution compared with I_o, that of the unirradiated one. Hence

$$D_t = \frac{kGE}{N} \times 10^7 \quad \text{.. .. ..} \quad (1)$$

To determine E we have to consider the decay of the radionuclide.

If A_o is the measured disintegration rate in disintegrations per second per c.c. at time t = o then the number of atoms disintegrating from zero time to time t is

$$N_o - N = \frac{A_o}{\lambda}(1 - e^{-\lambda t}) \text{ per c.c. solution.}$$

If $\overline{E}$ is the average energy in MeV dissipated per disintegration then

$$E = \frac{A_o \overline{E}}{\lambda}(1 - e^{-\lambda t}) \text{ MeV/c.c.} \quad \text{.. .. ..} \quad (2)$$

Now, substituting in equation (1)

$$D_t = \frac{k\ G\ A_0\ \overline{\overline{E}}}{N\lambda}\ (1 - e^{-\lambda t}) \times 10^7 \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (3)$$

and a plot of D_t against $(1 - e^{-\lambda t})$ should be a straight line through the origin with slope proportional to G.

The above deduction did not take into account any energy lost from the system by radiation through the walls of the container. The proportion of energy lost in this way is a function of the dimensions of the container and in the case of spherical flasks the fraction of energy lost would decrease with increasing radius. Accordingly, the true G-value can only be obtained by extrapolating the plot of the apparent G-value against r, the radius of the flask, to infinite radius, or what is usually easier, to extrapolate the plot against $\frac{1}{r}$ to zero.

Reagents and apparatus. Since the effect of organic impurities could be marked even if present in relatively small concentrations, it was decided to take all the necessary precautions to ensure a high degree of purity for the water used to prepare the "Fricke" solution. Water from the laboratory supply was distilled in an all-silica still, after which it was refluxed successively with alkaline potassium permanganate and acid sodium chromate solutions. The distillate from the chromate treatment was finally redistilled and this water was used to prepare the dosimeter solution and to wash all the apparatus which came into contact with the irradiation solution.

The sulphuric acid, ferrous ammonium sulphate and sodiu chloride used were all of Merck's "*pro analysi*" grade. The "Fricke" solution was made up in 2 l. batches, each batch containing 0·7845 g ferrous ammonium sulphate as hexahydrate, 0·1170 g sodium chloride and sufficient sulphuric acid to form a 0·8N solution. The solution was aerated with cleaned compressed air for about 24 hours before radioactive material was added.

Phosphorus-32 was obtained as carrier-free phosphate, in acid solution, from The Radiochemical Centre, Amersham, England. In the earlier irradiations specially prepared sulphuric acid solutions were ordered, but later batches were in hydrochloric acid and were converted to chloride-free sulphuric acid solutions by repeated evaporation with concentrated sulphuric acid in platinum-ware. The total amount of activity used for the irradiations varied between 125 and 250 mc.

The containers in which the irradiations were carried out (Fig. 1) were selected spherical glass flasks with a narrow constriction at the neck, so that the flask could be filled to the same volume every time. Samples of the irradiated solution were removed by a capillary-tipped pipette which passed through the constriction. The mean internal diameter of each of 22 flasks, ranging in capacity from about 6 to over 540 c.c., was determined by volume measurement and checked by direct measurements externally, corrected for wall thickness.

For the measurement of the ferric ion concentration the optical density of samples of the solution was determined in 1 cm silica cells at 304-305 mμ with a Unicam S.P. 500 spectrophotometer. As the temperature coefficient of the molar extinction coefficient is +0·7%, temperature control at 21·0 ± 0·2·C was sufficient.

The nuclear measurement equipment consisted of two type 532/A I.D.L. power supply units, two type 1009A and one 1009E Dynatron scalers, a Hewlett-Packard high speed decade scaler and one model 107P R.I.C. and one model N302 Hamner non-overleading linear amplifiers of the Chase-Higinbotham type. In addition, for scintillation counting the photomultiplier was an E.M.I. type 6097S tube and was used with a liquid scintillator solution consisting of a mixture of 82 parts toluene and

18 parts ethyl alcohol as solvent, and primary and secondary solutes respectively, 14 g/l. P.P.O. and 0·5 g/l. P.O.P.O.P. as supplied by Messrs. Nuclear Enterprises Ltd.

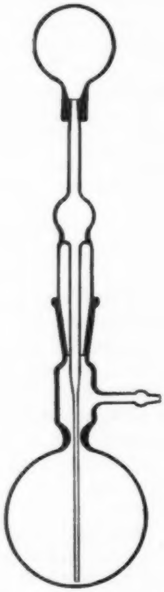


Fig. 1—Irradiation flask showing the capillary-tipped pipette with its rubber bulb attached, in position. For irradiation under aeration the rubber bulb is removed and the air supply is attached instead

SUPPLEMENTARY DETERMINATIONS

(1) *Calibration of absorption cells.* A total of 16 1 cm silica absorption cells were calibrated relative to one another by comparing the intensity of transmitted light of the same "Fricke" solution at 304-305 mμ. All later measurements made with these cells were corrected accordingly.

(2) *Determination of the molar extinction coefficient* (*k*). Several determinations of k were made with standardised ferric solutions in 0·8N sulphuric acid. The concentration of ferric iron ranged from zero to 0·45 mM in a solution with total iron concentration of 1·000 mM. The value obtained is in good agreement with previously reported values, as is shown in Table I.

(3) *Purity of the emitter.* Carrier-free radiophosphorus is produced by neutron irradiation of sulphur. Since natural sulphur consists mainly of the isotope S^{32} with about 0·75% S^{33} and 4·2% S^{34}, it may be expected that the (n, p) reaction which produces phosphorus-32 should be accompanied by a similar reaction producing phosphorus-33. The half-life of phosphorus-34 is so short (12·4 seconds) that it need not be taken into account. Both phosphorus-32 and 33 are β^-emitters and their half-lives are 14·3 days and 25 days respectively, so that both these isotopes will be present for the duration of the irradiation experiment.

TABLE I

The molar extinction coefficient of ferric iron

Reference	k reported [mole^{-1} cm^{-1}]	Temp. [°C]	k calc. 21°C [mole^{-1} cm^{-1}]
11	2150	20	2165
12	2094	20	2109±20
1	2174	23·7	2133
6	2196	25	2135±6
		This work	2122±9

The purity of the radioactive emitter was confirmed and the relative amounts of the two isotopes present were determined by means of the isotope separator of the Nuclear Physics Division of the National Physical Research Laboratory. A sample of the radioactive solution, as received, was added to about 1 g of chemically pure phosphoric acid and heated *in vacuo* to about 300°C to remove most of the moisture. The residue was transferred to the sample holder of the isotope separator and analysed. Radioactivity was found corresponding to a large variety of mass-charge ratios, but the series of lines between mass-charge ratios of 47 and 51 proved most convenient for analysis. A typical analysis of the radioactivity of this group is shown diagrammatically in Fig. 2. The shaded portions represent the activities of strips cut out from the target receiver plate and measured. The activity of mass 48 was found to contain only phosphorus-32, presumably as $[P^{32}O^{16}]^+$, whereas the activity at mass 49 contained comparable proportions of phosphorus-32 and 33. From these two lines the isotopic composition of the radioactivity was determined.

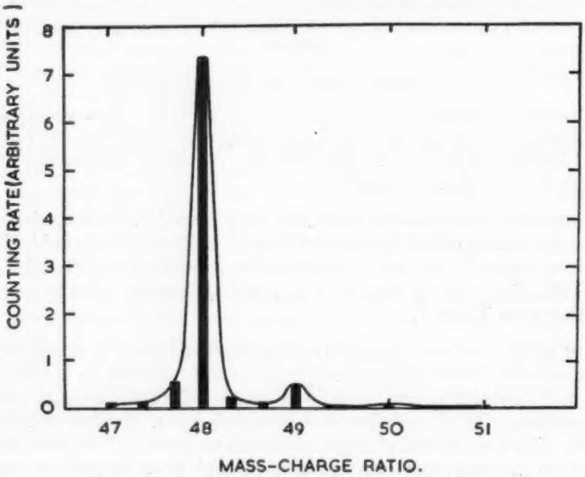


Fig. 2—Isotopic analysis of the radioactive emitter

The strips corresponding to masses 48 and 49 were counted under identical conditions and the absorption of the β^- radiations in aluminium was determined. Using the absorption data of mass 48, which contained only phosphorus-32, the absorption data of mass 49 was corrected to give the relative concentration of phosphorus-33. It was found that 1·6 ± 0·16 per cent (estimated total error) of the activity was due to the heavier isotope. If it is assumed that the relative average energies dissipated per disintegration of the two isotopes are in the ratios of their respective maximum energies, then the calculated energy dissipated by the mixture is about 98·6% of that dissipated by pure phosphorus-32 with the same total activity. Since the two isotopes would both contribute to the counting rate, A_o, the total energy, E, absorbed by the system as given by equation (2) has to be corrected for the phosphorus-33 content. In all results given subsequently this correction has been made.

(4) *The relationship between* D_t *and* $(1 - e^{-\lambda t})$. To establish that the ferric ion concentration, as measured by the optical density of the solution, D_t, increased linearly with $(1 - e^{-\lambda t})$ one irradiation experiment was carried out in which measurements were taken periodically for each of 22 flasks without interruption over some 190 hours. The results showed that for the larger flasks D_t increased linearly with the decay of phosphorous-32. However, for smaller flasks there was an apparent deviation from linearity, in the plot of D_t against $(1 - e^{-\lambda t})$. This effect was due to the fact that for the smaller flasks the volume of solution removed for measurement represented an appreciable proportion of the total volume. Consequently, when a large number of measurements was made there was an appreciable change in the geometrical shape of the liquid during its irradiation and hence the fraction of energy escaping from the solution, and therfore its radiation dose rate could no longer be assumed directly proportional to $(1 - e^{-\lambda t})$ over the entire irradiation period. In order to prevent this effect, reliable results could be obtained when the number of samples taken for measurement from the smaller flasks was kept as small as was consistent with the accuracy required.

(5) *The effect of oxygen concentration.* During irradiation a stage will eventually be reached where there is a marked decrease in the G-value due to oxygen deficiency. To show that, under the conditions described in this publication, this stage was never reached, an experiment was carried out where two parallel series of solutions were irradiated in which one was left undisturbed whilst the other was continuously aerated with a very slow stream of air. It was found that the G-values determined on the undisturbed solutions were not significally different from those of the aerated ones.

Procedure. Immediately prior to irradiation several samples of the "Fricke" solution were removed for use as reference solutions in the optical measurements. The addition of the carrier-free phosphorus-32 marked the beginning of the irradiation. The solution was then dispensed into the spherical irradiation flasks as quickly as possible and left in a thermostatically controlled bath at 21°C. Small samples of the solution were removed from the larger flasks at various times for absolute measurement of radioactivity.

The solutions as drawn from the flask had too high an activity to be counted directly, and were diluted with distilled water containing 200 mg/l. of phosphate carrier as NaH_2PO_4. From each of these diluted solutions a large number of sources were prepared for both the 4π-proportional and the 4π-scintillation counting methods.

For proportional counting, sources were prepared as almost weightless deposits on thin films of cellulose acetate made conducting by the vacuum evaporation of gold on to it. The sulphuric acid in the sample had to be neutralised with ammonia gas to prevent the acid attacking the film. Counting was carried out in the same N.P.R.L. 4π-proportional counter normally used for international comparisons of radioactivity standards.[14] For liquid scintillation counting samples of the diluted solutions were weighed out and transferred to the counting cells by means of small weighing pipettes, mixed with 12 c.c. liquid scintillation solution and counted by the new 4π-scintillation counting method developed in this Laboratory[7].

The growth of ferric ion concentration during irradiation was followed spectrophotometrically. Measurements were made in the absorption peak of ferric ions at 304-305 mμ and it was found convenient to make the first set of measurements some 5 to 7 days after the start, depending on the level of activity used. Thereafter measurements were taken periodically over a period of about a month. Two aliquots were removed from each flask and their optical density measured in the calibrated silica cells against samples from two of the reference solutions. In this way errors due to differences in the cells, possible contamination of the reference solution, and other such sources were reduced. After measurement, the samples were returned to the flasks so that the spherical geometry of the irradiation mixture could be retained. The mean value of D_t from all these determinations and the corresponding value of $(1 - e^{-\lambda t})$ were used in equation (3) to calculate the apparent G-value.

RESULTS AND DISCUSSION

The linear relationship between D_t and $(1 - e^{-\lambda t})$ is shown in Fig. 3. It is now possible to calculate an apparent G-value for each flask, provided that the constants in equation (3) and the particular value of A_0 for the irradiation experiment are known. The constants used to calculate all the results in this investigation are:

$N = 6{\cdot}025 \times 10^{23}$ atoms per g mole (Avogadro number)
$\lambda = 5{\cdot}61 \times 10^{-7}$ sec^{-1} corresponding to a half-life of 14·3 days
$k = 2122 \pm 9$ mole^{-1} cm^{-1} at 21°C (*vide supra*)
$\overline{E} = 0{\cdot}690$ MeV (calculated — *vide infra*)

The value of $\overline{E}$ has been calculated[9] from the experimentally determined maximum value of β^- energy and an assumed allowed shape of the beta spectrum. Several experimental determinations have been made[9] and the results are in fair agreement with this value; accordingly the calculated value is used here. Any error inherent in this value will also be reflected in the G-value valculated from it. Should a better value be determined in the future, the results given below will have to be adjusted accordingly.

The results of the absolute standardisation of radioactivity, A_0, for the different experiments are given in Table II and the errors listed therein are the standard errors (given by $\frac{\sigma}{\sqrt{n}}$). The ratio of the activities as found by the two standardisation methods has a mean value of 1·0114, *i.e.*, the 4π-liquid scintillation counting method gives results about 1·14% higher than the 4π-proportional counting method. This discrepancy is consistent and quite reproducible[7]. To decide between the results of the two methods is not straightforward. For some years now it was believed that the proportional counting method had an efficiency of very nearly 100% for high energy beta emitters, such as phosphorus-32[10]. On the other hand, the liquid scintillation counting method is a new development still lacking the status of the older method. However, recent work[8] has produced evidence to show that the efficiency of proportional counting of phosphorus-32 may not be as high as was believed. By incorporating two different radionuclides in the same compound, one of which could be standardised by the β–γ coincidence counting method, it was shown[8] that the self-absorption for phosphorus-32 amounted to about 1%. It is thus possible that of the two methods used here the 4π-liquid scintillation counting method may be the more accurate after all.

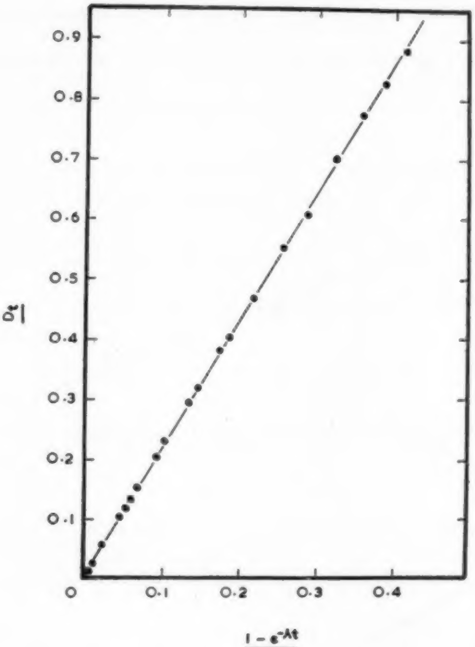


Fig. 3—The variation of the optical density with the decay of the P^{32}

TABLE II

Absolute standardisation of radioactivity

Experiment	Proportional counting (P) [sec^{-1} c.c.$^{-1}$]	Scintillation counting (S) [sec^{-1} c.c.$^{-1}$]	Ratio $\frac{S}{P}$
1	$3{\cdot}4826 \pm 0{\cdot}0075 \times 16^{6}$	$3{\cdot}5299 \pm 0{\cdot}0037 \times 10^{6}$	1·0136
2	$3{\cdot}4495 \pm 0{\cdot}0034$	$3{\cdot}4882 \pm 0{\cdot}0009$	1·0112
3	$3{\cdot}9522 \pm 0{\cdot}0037$	$3{\cdot}9787 \pm 0{\cdot}0037$	1·0067
4	$1{\cdot}9905 \pm 0{\cdot}0018$	$2{\cdot}0187 \pm 0{\cdot}0020$	1·0142
5	$2{\cdot}6698 \pm 0{\cdot}0026$	$2{\cdot}7000 \pm 0{\cdot}0034$	1·0113
		Mean ratio	1·0114

The apparent G-values, G_r, obtained for each flask, are plotted against $\frac{1}{r}$ in Fig. 4 and the errors shown are standard errors. The straight line in the figure is that calculated by the method of "least squares", each point being given a statistical weight in the calculation according to the number of determinations made with the corresponding flask. G_r, for the zero value of $\frac{1}{r}$, *i.e.*, an infinite flask, is obtained from the

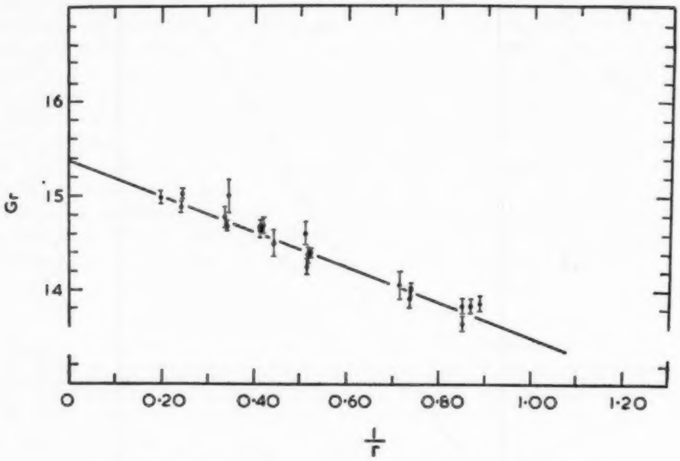


Fig. 4—Variation of apparent G-value with radius^{-1}

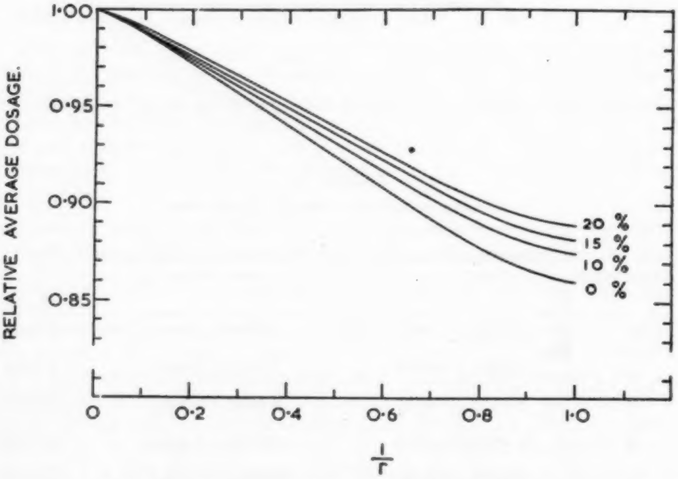


Fig. 5—The average dosage inside a spherical uniform beta source relative to that inside an infinite sphere, after Hine and Brownell.[9] The curves are drawn for 0, 10, 15 and 20% back-scatter

straight line and the corresponding error is the "95% error" calculated from the same data; this value of G_r is the G-value of the radiation reaction.

The assumption of a linear relationship between G_r and $\frac{1}{r}$ requires justification. The problem of determining the average radiation dosage inside a spherical uniform beta source has been treated by Hine and Brownell,[9] from whose data Fig. 5 has been prepared, showing the variation with radius of the ratio of the average radiation dosage inside a sphere of radius r to that inside an infinite sphere. The effect of back-scatter from the walls of the container is shown by the curves of Fig. 5 drawn for 0, 10, 15 and 20% back-scatter.

When these curves are made to coincide with the value of the calculated straight line of Fig. 4 at $\frac{1}{r} = 0{\cdot}2$, *i.e.*, near the value corresponding to the largest flask used in the experiment, it is found that the values obtained from the 15 and 20% back-scatter curves (Fig. 5) do not differ appreciably from the corresponding values from the straight line (Fig. 4) for $\frac{1}{r}$ from zero to unity. This relationship is shown diagrammatically in Fig. 6.

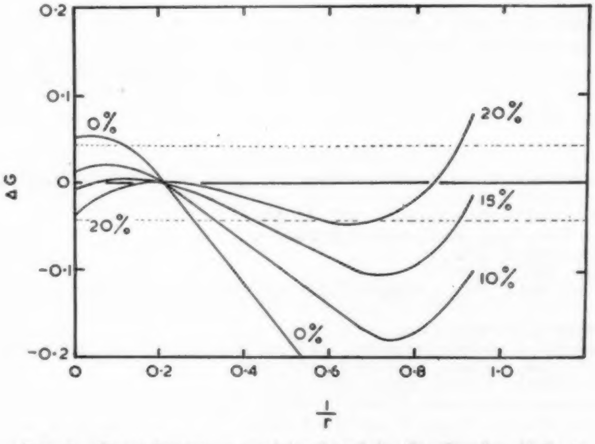


Fig. 6—The inherent error introduced in the G-value if G_r is assumed to vary linearly with $\frac{1}{r}$ (see text). For comparison the dotted lines show the 95% error of G at $\frac{1}{r}=0$

If G_1 is the value of G_r obtained from the straight line at any value of $\frac{1}{r}$ and G_C is the corresponding value obtained on the basis of the curves in Fig. 5 then

$$\Delta G = G_C - G_1$$

represents the inherent error made by assuming a linear relationship between G_r and $\frac{1}{r}$. Fig. 6 shows the plot of ΔG against $\frac{1}{r}$. It is clear that ΔG is within the experimental error for almost the entire range of $\frac{1}{r}$ less than unity, if the back-scatter contribution is between 15 and 20%. This value for the back-scatter contribution also agrees with the value of 15% found[13] from measurements with aluminium, and it is concluded that a linear relationship between G_r and $\frac{1}{r}$ is justified.

The radiolytic yield of ferric ions in the "Fricke" solution was thus found to be 15.39 ± 0·04 ions per 100 eV by proportional counting and 15·21 ± 0·04 by liquid scintillation counting. As these values depend on that of $\overline{E}$ they may be given as 10·62 $\overline{E}^{-1}$ and 10·50 $\overline{E}^{-1}$ respectively.

The authors acknowledge their appreciation to Dr. W. E. Frahn and his co-workers for their assistance with the isotopic analysis of the radioactive material and to D. van As, H. L. Lawrenz, P. J. Horn and Mrs. M. M. Pols for technical assistance.

†Radiochemistry Section,
National Chemical Research Laboratory,
S.A. Council for Scientific and Industrial Research,
Pretoria.

‡Radioactivity Division,
National Physical Research Laboratory,
S.A. Council for Scientific and Industrial Research,
Pretoria.

Received January 2, 1960.

REFERENCES

[1] Weiss, Allen and Schwarz, *International Conf. Peaceful Uses of Atomic Energy*, Geneva, 1955, P/155.
[2] Hardwick, *Discussions Faraday Soc.*, 1952, **12**, 205.
[3] Hart, *Annual Rev. Phys. Chem.*, 1954, **5**, 142 *et seq.*
[4] Dainton, *ibid.*, 1955, **5**, 215.
[5] Garrison, *ibid.*, 1957, **8**, 150.
[6] Brynjolfson and Holm, *Proc. Symposium Metrology Radionuclides*, Vienna, 1959. In the press.
[7] Steyn, *idem.*
[8] Campion, Taylor and Meritt, *idem.*
[9] Hine and Brownell, *Radiation Dosimetry*, Academic Press, New York, 1956, p. 694 *et seq.*
[10] Pate and Yaffe, *Can. J. Chem.*, 1955, **33**, 15, 925, 1656.
[11] Donaldson and Miller, *J. Chim. Phys.*, 1955, **52**, 578.
[12] Swallow, Discussion on reference 11, *ibid.*, 1955, **52**, 585.
[13] Failla, *Progress Nuclear Energy*, Series VII, 1956, **1**, 156.
[14] *Standards of physical measurement and means for their utilisation, as well as other testing facilities available in the South African National Physical Research Laboratory*, C.S.I.R. publication, in the press.

THE BITTER PRINCIPLE OF *SOLANUM MELONGENA* (EGG PLANT) FRUITS

by

H. L. DE WAAL, L. P. NEETHLING and G. W. PEROLD

OPSOMMING

Die bitterstof van ryp vrugte van *Solanum melongena* is 'n solasonien wat identies is met solasonien wat in *Solanum sodomaeum* voorkom.

SUMMARY

The bitter principle of ripe fruits of *Solanum melongena* is a solasonine which is identical with solasonine occurring in *Solanum sodomaeum.*

The only report to date on the bitter constituents of Egg Plant (*Solanum melongena*) fruits,[1] describes the isolation of a glyco-alkaloid, solanine M, $C_{31}H_{51}NO_{12}$. Locally grown ripe fruits of a bitter strain of this plant have now afforded a very low yield of a glyco-alkaloid, $C_{45}H_{73}NO_{16}\cdot 3H_2O$, m.p. 265-275° (dec.) as the only isolable alkaloidal constituent. This is a solasonine, and not a solanine compound.

This glyco-alkaloid showed infrared absorption identical with that of authentic solasonine,* obtained from *Solanum sodomaeum*, and differing from that of solanine (Merck)† in having peaks at 897, 882 and 835 cm^{-1} while lacking the peak at 823 cm^{-1}.

Hydrolysis of the glyco-alkaloid yielded the aglycone hydrochloride, $C_{27}H_{43}NO_2.HCl.\frac{1}{2}H_2O$ ‡, m.p. 265-270° **, together with the three sugars, galactose, glucose and rhamnose which occur also in solasonine and in solanine.[6] The well-developed infrared absorption spectrum of the aglycone hydrochloride was in every way identical with that of solasodine hydrochloride, the peak at 3356 cm^{-1} (NH), absent in solanidine hydrochloride, being fully developed next to the peak at 3280 cm^{-1} (OH) common to both. The free aglycone base was furthermore examined by paper chromatography, when its behaviour was identical with that of solasodine, while differing significantly from that of solanidine.

The optical rotations found for the present glyco-alkaloid and its aglycone hydrochloride, although they could not here (see Experimental) be determined accurately, agreed within rather wide limits with values for solasonine and solasodine hydrochloride respectively.

---

*This reference sample was isolated by Professor L. H. Briggs, Auckland University College, and gratefully received through the courtesy of Dr. D. P. Veldsman, formerly of the Veterinary Research Laboratory, Onderstepoort, and Dr. P. R. Enslin, National Chemical Research Laboratory, Pretoria[2].

†Professor C. Schopf, Darmstadt, is thanked for his kind help in obtaining this sample.

‡The same composition was found for the hydrochloride of solasodine by Briggs *et al.*[3]

**The m.p. of a reference sample of authentic solasodine hydrochloride was here found as *ca* 260°, as against the reported value[3] of 314°. This stresses the unreliability of m.p.s and mixed m.p.s as criteria of identity in this field.[4,5]

The complete identity of the infrared absorption of the aglycone hydrochloride over the range 2-15μ with that of solasodine hydrochloride shows that the aglycone isolated is unlikely to be a C_{22}- or C_{25}- epimer of solasodine, as pairs of such epimers in the related field of the sapogenins[*] show slight but quite definite differences in the 900 cm^{-1} region.[7]

The glyco-alkaloid now isolated from *Solanum melongena* is therefore defined as solasonine itself, containing the aglycone solasodine and a trisaccharide composed of galactose, glucose and rhamnose.

EXPERIMENTAL

Infrared absorption spectra were obtained in potassium bromide dispersions. Optical rotations were measured at room temperature. M.p.s were taken on a Kofler block.

The glyco-alkaloid. Bitter[†] fruits of *Solanum melongena* were harvested two weeks after ripening, air-dried, powdered and extracted with 1-2 l. of 2% aqueous acetic acid per 100 g powder for 24 hours at 50°. Ammonia was added to the filtered extract and the precipitate collected and washed in the centrifuge. The dried precipitate was exhaustively extracted (Soxhlet app.) with 96% ethanol, the extract dried, taken up in 1% aqueous hydrochloric acid and filtered. The alkaloids were precipitated with excess of Mayer's potassium mercuric iodide reagent, the precipitate suspended in water and mercury removed by treating with hydrogen sulphide. On adding ammonia to the filtered solution and warming, the crude glyco-alkaloid was obtained and purified repeatedly by treating with animal charcoal in ethanolic solution. Six-fold slow crystallisation from methanol-water (4:1) afforded the pure glyco-alkaloid as fine needles, m.p. 265-275° (dec.), $[\alpha]_D$-71°±25°[‡] (c, 0·97% in MeOH, 5 cm microtube). The isolation of the pure product was always difficult and the final yield never exceeded 0.00005%, based on fresh fruit. For analysis a sample was dried and then equilibrated in air (Found for separate preparations: C, 57·3; H, 8·4; N, 1·6, 1·8. Calc. for $C_{45}H_{73}NO_{16}.3H_2O$: C, 57·6; H, 8·5; N, 1·5%).

The aglycone hydrochloride. The glyco-alkaloid (40 mg) was refluxed for 4 hours in ethanol (1·6 ml) and ION aqueous hydrochloric acid (0·4 ml) to give a quantitative yield of the insoluble aglycone hydrochloride (19 mg). Four crystallisations from methanol containing a trace of hydrochloric acid gave the aglycone hydrochloride as colourless, nonhygroscopic needles, m.p. 265-270°, $[\alpha]_D$-92°±25°[**] (c, 0·26% in MeOH, 5 cm microtube), (Found for two separate preparations: C, 70·7; H, 10·0; C1, 7·8; N, 2·8. Calc. for $C_{27}H_{43}NO_2.HC1.\frac{1}{2}H_2O$: C, 70·6; H, 9·9; C1, 7·7; N, 3·1%).

Paper chromatography of the aglycone. The free aglycone was obtained from the aglycone hydrochloride (1 mg) by treatment with potassium hydroxide in hot methanolic solution. After drying, the free aglycone was taken up in 0·1 ml chloroform; spots of 100 μg each were chromatographed following the method of Tuzson.[11]

---

[*] In our hands such differences were clearly shown by the infrared absorption spectra of the C_{25}-isomeric sapogenins, ruscogenin and neo-ruscogenin, obtained on samples kindly provided by Mr. W. T. de Kock, National Chemical Research Laboratory, Pretoria.

[†] Private communication from Dr. S. Rehm, 9/10/59:— Propagation experiments have shown that bitterness in these fruits is due to a mutation within the species, and not to hybridisation with any of the indigenous wild *Solanum* species.

[‡] Values of m.p. 285° and $[\alpha]_D$-74·5(MeOH) have been reported.[8,9] The unreliability of m.p. data in this field has already been stressed.

[**] The rotation of a reference sample of solasodine hydrochloride, similarly prepared from solasonine ex *Solanum sodomaeum* was here found as $[\alpha]_D$-96°±25° (c, 0·28% in MeOH, 5 cm microtube), as against a reported value of $[\alpha]_D$-68°.[10]

Antimony trichloride reagent gave spots displaying the same colour and fluorescence in ultraviolet light as shown by solasodine under the same conditions. $R_{\text{solanidine}}$ values were calculated relative to simultaneously run samples of solanidine (Found: $R_{\text{solanidine}}$, **1·10±0·01**. $R_{\text{solanidine}}$ for solasodine, 1·09±0·01).

The sugars. The available amounts of the glyco-alkaloid allowed only the paper chromatographic identification of the sugars in the filtrate obtained in the preparation of the aglycone hydrochloride (above). Only by following the method of Chargaff *et al.*[12] could galactose and glucose be obtained as distinct spots. The system pyridine: water:n-butanol (1:1·5:3) was used, an equal volume of pyridine being added to the upper phase. Spots were located by spraying with aniline phthalate in n-butanol, followed by drying the paper for 5 mins. at 105°. In all chromatograms, the three spots were of the same size. The $R_{\text{galactose}}$-values found for the spots were 1·00± 0·01, 1·09±0·01 and 1·38±0·01, as against the values for galactose (=1), glucose (1·09) and rhamnose (1·38).

We are greatly indebted to Dr. S. Rehm of the Horticultural Research Station of the Union Department of Agriculture, for his continued interest in growing and providing the plant material. One of us (L.P.N.) thanks Dr. F. G. Anderssen, Head of the Division of Horticulture, Pretoria, for permission to carry out this work.

Department of Organic Chemistry,
University of Pretoria.

Division of Organic Chemistry,
Research and Process Development Department,
ISCOR,
P.O. Box 450,
Pretoria.

Received January 23, 1960.

REFERENCES

[1] Minasyan, *Biokhimiya*, 1947, **12**, 298.
[2] Briggs, Newbold and Stace, *J. Chem. Soc.*, 1942, 9.
[3] Bell and Briggs, *ibid.*, 1942, 1.
[4] Bell, Briggs and Carroll, *ibid.*, 1942, 12.
[5] Veldsman and Louw, *J. S. African Chem. Inst.*, 1949, **2**, 120.
[6] Prelog and Jeger, *The Alkaloids*, edited by Manske and Holmes, Academic Press Inc., New York, 1953, Vol. III, p. 249.
[7] Callow, Dickson, Elks, Evans, James, Long, Oughton and Page, *J. Chem. Soc.*, 1955, 1969.
[8] Prelog and Jeger, *The Alkaloids*, edited by Manske and Holmes, Academic Press Inc., New York, 1953, Vol. III, p. 252.
[9] Kuhn and Löw, *Chem. Ber.*, 1955, **88**, 292.
[10] Bell and Briggs, *J. Chem. Soc.*, 1942, 2.
[11] Tuzson, *Naturwiss.*, 1956, **43**, 198.
[12] Chargoff, Levine and Green, *J. Biol. Chem.*, 1948, **175**, 70.

COMPLEX CYANIDES OF TRIVALENT GOLD

by

P. O. FINSEN AND K. A. MURRAY

OPSOMMING

Eksperimente is uitgevoer waarin gebruik gemaak is van 'n aantal fisies-chemiese tegnieke, bv. potensiometrie met die gebruik van goud en platina elektrodes in neutrale en suuroplossing, interne elektrolise in alkaliese oplossing en kompleksimetriese titrasie met ,,EDTA" in ammoniak-oplossing. Dit word afgelei dat die sianiedekwivalent van trivalente goud afhanklik is van die pH-waarde van die oplossing.

SUMMARY

Experiments have been carried out employing a number of physico-chemical techniques, e.g. potentiometry using a gold and a platinum electrode in neutral and acid solution, spontaneous or internal electrolysis in alkaline solution and complexometric titrations with "EDTA" in ammoniacal solution. It is concluded that the cyanide equivalent of trivalent gold is dependent on the pH value of the solution.

INTRODUCTION

Murray[1] showed that when aqueous gold trichloride or chloroauric acid ($AuCl_3$ and $HAuCl_4$ respectively) was titrated with potassium cyanide, a rapid change in the potential of a platinum electrode immersed in the solution took place after a definite molecular proportion of potassium cyanide had been added. Using chloroauric acid in subsequent investigations, the authors found that a gold electrode exhibited similar behaviour to that of the platinum electrode, the change in potential being more pronounced. This effect with the gold electrode could be enhanced by excluding atmospheric oxygen from the system, a drop in potential of as much as 1·3 volt being obtained in the potentiometric titration of a 0·01M solution of chloroauric acid. The platinum electrode was unaffected by atmospheric oxygen. These findings are illustrated by the titration curves in Fig. 1, which were obtained by titrating 0·001M chloroauric acid with potassium cyanide in the presence and in the absence of atmospheric oxygen, using both gold and platinum electrodes.

(*Note*: All potentials reported in this publication are referred to the saturated calomel electrode).

EXPERIMENTAL AND RESULTS

Design and construction of a semi-micro potentiometric titration cell. In order to avoid the use of excessively dilute solutions the titration cell was constructed as small as practicable, since on account of the cost of gold the solutions had to be used frugally. The cell was made from a B24 socket, the plain end being closed. A thick-walled capillary and a microporous porcelain plug were sealed into the bottom of the cell as shown in Fig. 2. The porcelain for the porous plug was specially prepared from medicinal kaolin and the plug was carefully sealed into the borosilicate glass cell.

The gold wire electrode was fixed rigidly into a B24 cone by means of plaster of Paris, a short glass tube also being cast in to accommodate the tip of a burette. The lower end of the cell was immersed in a small vessel containing potassium chloride

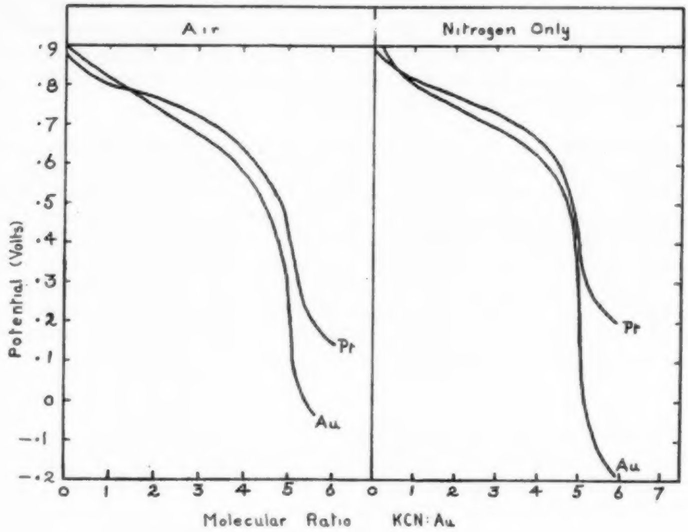


FIG. 1—Titration of $HAuCl_4$ with KCN

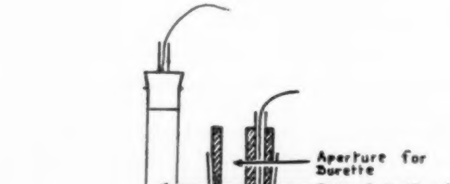

Aperture for Burette
Calomel Half-cell
HAuCl4 Solution
Gold Wire
Porous Plug
KCl Solution

FIG. 2—Diagram of semi-micro potentiometric titration cell

solution, electrolytic contact thereby being made with a saturated calomel electrode. Similar provision was made for the use, if necessary, of a platinum electrode in place of the gold. Nitrogen (or air, if desired) could be introduced by means of the capillary tube, thereby also keeping the solution efficiently stirred. Quantities as small as 2 ml could be titrated conveniently with this apparatus.

Potentiometric titration in acid and neutral solutions. Results obtained with the gold and platinum electrodes indicated that four molecules of potassium cyanide were equivalent to one molecule of gold trichloride, while five molecules of potassium cyanide were equivalent to one molecule of chloroauric acid. Furthermore, a mixture of equimolecular quantities of gold trichloride and hydrochloric acid behaved in the same way as chloroauric acid, while any further addition of hydrochloric acid resulted in the potassium cyanide titre being increased in the ratio of one gram molecule of potassium cyanide to one gram molecule of hydrochloric acid. Similarly, a mixture of equimolecular quantities of chloroauric acid and potassium hydroxide behaved in the same way as gold trichloride, thus:

$$\begin{aligned}
&\mathrm{AuCl_3} & & \equiv 4\,\mathrm{KCN}\\
&\mathrm{HAuCl_4} & + \mathrm{KOH} & \equiv 4\,\mathrm{KCN}\\
&\mathrm{AuCl_3} & + \mathrm{HCl} & \equiv 5\,\mathrm{KCN}\\
&\mathrm{HAuCl_4} & + \mathrm{HCl} & \equiv 6\,\mathrm{KCN}\\
&\mathrm{HAuCl_4} & + 4\mathrm{HCl} & \equiv 9\,\mathrm{KCN}
\end{aligned}$$

Raines[2] prepared cyanoauric acid by passing hydrogen cyanide into aqueous gold trichloride.

$$\mathrm{AuCl_3} + 4\mathrm{HCN} \longrightarrow \mathrm{HAu(CN)_4} + 3\,\mathrm{HCl}$$

As a working hypothesis it was assumed that the cyanoaurate ion was also formed in the reactions giving rise to the above equivalences, thus:

$$\mathrm{AuCl_3} + 4\mathrm{KCN} \longrightarrow \mathrm{Au(CN)_4}^- + 4\mathrm{K}^+ + 3\mathrm{Cl}^-$$

$$\begin{aligned}
\mathrm{HAuCl_4} + \mathrm{nHCl} + (\mathrm{n}+5)\mathrm{KCN} \longrightarrow{}& \mathrm{Au(CN)_4}^- + (\mathrm{n}+5)\mathrm{K}^+\\
&+ (\mathrm{n}+4)\mathrm{Cl}^- + (\mathrm{n}+1)\mathrm{HCN}
\end{aligned}$$

The stoicheiometry of the latter equation was confirmed in the following manner. A mixture of 0·01M chloroauric acid (5 ml), 0·1N hydrochloric acid (10 ml) and 0·1M potassium cyanide (7·5 ml) was scrubbed with carbon dioxide-free air, which was then passed through a known volume of standard alkali. Any excess cyanide over and above that required for the complexing of the gold would appear in the alkali in the form of alkali cyanide, having passed over in the air stream as hydrocyanic acid. Determinations of the cyanide concentration in the alkali solution after varying time intervals indicated that after one day's scrubbing the ratio of cyanide to gold in the reaction mixture was approximately 4. Protracted scrubbing for 25 days resulted in the loss of a small further quantity of cyanide from the reaction mixture, but this is not regarded as significant.

The addition of alkali (e.g. sodium hydroxide) to chloroauric acid in excess of one equivalent of alkali per molecule of chloroauric acid prior to titration with potassium cyanide, resulted in a pronounced "drifting" of the potential exhibited by the gold electrode. Fig. 3 shows the titration curves obtained when chloroauric acid treated, with the indicated molecular ratios of acid and alkali, is titrated with potassium cyanide. The broken line indicates the effect of allowing 5 minutes to elapse after each addition from the burette before measuring the potential.

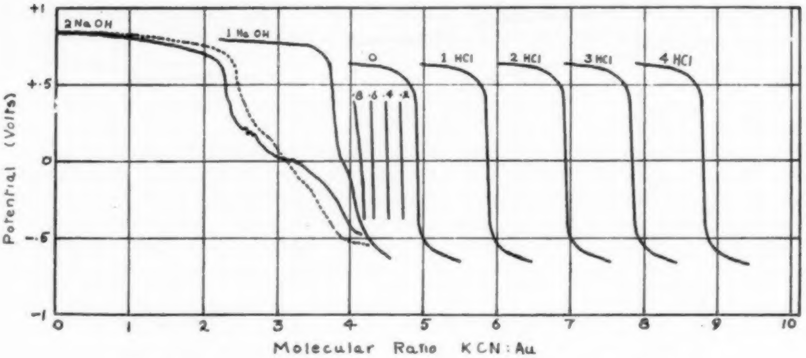


FIG. 3—Titration of $HAuCl_4$ containing alkali or acid with KCN

The effect of potassium chloride on the reaction. In an attempt to gauge the stability of the cyanoaurate ion, 2M potassium chloride (1 ml) was added to 0·01M gold trichloride (5 ml) prior to titration with potassium cyanide. It was expected that the equilibrium should be driven to the left by the large excess of chloride ion:

$$AuCl_3 + 4(CN)^- \rightleftharpoons (Au(CN)_4)^- + 3\ Cl^-$$

However, an increase in the cyanide titre of only 2 per cent confirmed qualitatively that the chloride ion is much more loosely bound to the gold atom than is the cyanide ion.

The titration of hydrochloric acid with potassium cyanide. When hydrochloric acid was titrated with potassium cyanide in the absence of any gold compound in solution, the gold electrode exhibited a sharp change in potential after the addition of 1 gram molecule of potassium cyanide per gram molecule of hydrochloric acid (Fig. 4, curve 1). The exclusion of atmospheric oxygen resulted in a curve of similar shape but slightly displaced in the direction of negative potential (curve 2). Under the same conditions the platinum electrode showed only a very small change in potential, and was not affected by atmospheric oxygen (curve 3, 4).

It is clear that after all the hydrochloric acid has reacted with potassium cyanide to produce feebly-ionized hydrocyanic acid, further addition of potassium cyanide caused a rapid rise in cyanide ion concentration. Under these conditions the gold electrode tended to be attacked according to the equation:

$$Au^\circ + 2CN^- \longrightarrow Au(CN)_2^- + e$$

This accounts for the drop in the potential of a gold electrode at the first appearance of excess alkali cyanide. In a similar way, the very small change in potential of a platinum electrode under the same conditions reflects the comparative resistance of platinum to attack by cyanide.

Products of the reaction. The following alternative equations had been considered initially as possibly representing the reaction between gold trichloride and potassium cyanide:

$$AuCl_3 + 4KCN \longrightarrow KAu(CN)_2 + 3KCl + (CN)_2$$

$$AuCl_3 + 4KCN + H_2O \longrightarrow KAu(CN)_2 + 3KCl + HCN + HCNO$$

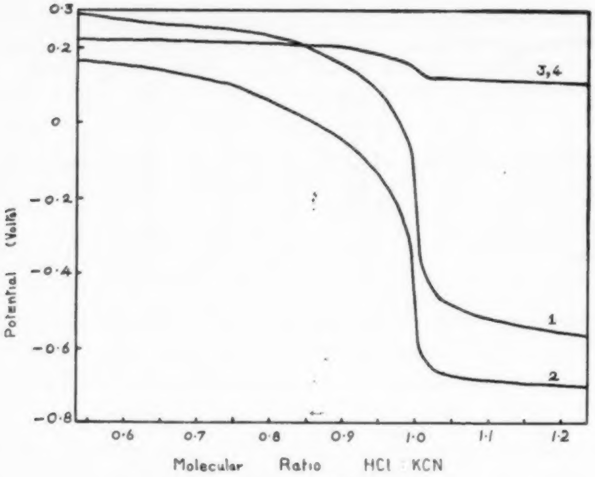


FIG. 4—Titration of HCl with KCN

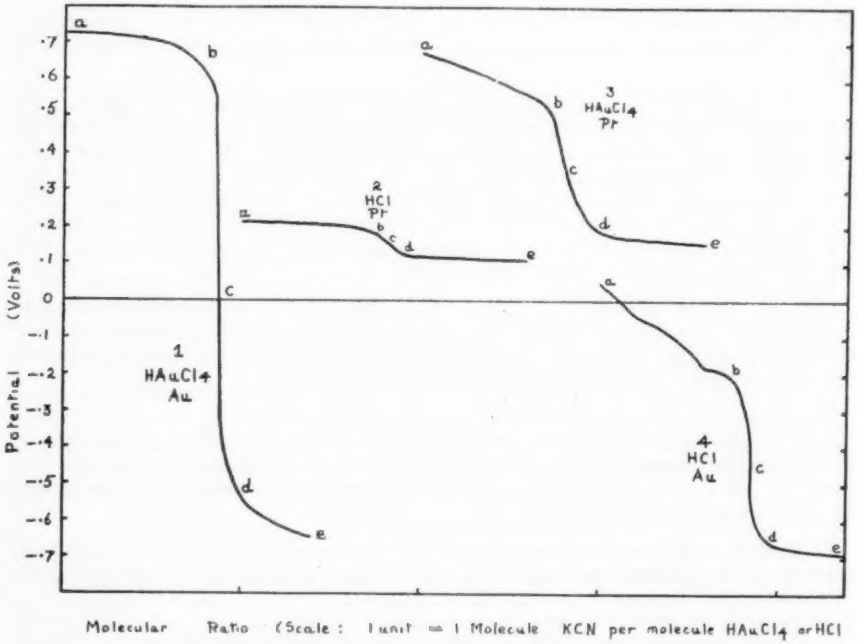


FIG. 5—Tritation of $HAuCl_4$ and HCl with KCN using gold and platinum electrodes

However, repeated tests for cyanogen and cyanate gave negative results. It became clear then that the reaction was exclusively one of complexing, no oxidation or reduction taking place.

It has been stated[3] that the tendency of trivalent gold to form complexes is so great that the simple ion Au^{3+} does not exist. However, Au^{3+}, the implied aqua complex, must theoretically be capable of existence, however evanescent, in solution in equilibrium with its other complex ions. In any case, equilibrium between an element and its ions is implicit in the concept of a standard electrode potential, that of gold being quoted[4] as +1·42V. Therefore, it was concluded that the standard gold electrode potential is dependent on the following equilibria, the first of which was studied by Bjerrum,[5] who obtained the following values for the hydrolysis constants of chloroauric acid:

$$K_1 = 10^{-6 \cdot 07},\ K_2 = 10^{-7},\ K_3 = 10^{-8 \cdot 06},\ K_4 = 10^{-8 \cdot 51}$$

$$AuCl_4^- + 2H_2O \rightleftharpoons AuO_2^- + 4HCl \text{ (in 4 stages)}$$

$$AuO_2^- + 2H_2O \rightleftharpoons Au^{3+} + 4OH^-$$

$$Au^{3+} + 3e \rightleftharpoons Au^0$$

As a result it is seen that as the titration of chloroauric acid with potassium cyanide approaches the value of five gram molecules of potassium cyanide per gram molecule of chloroauric acid, the concentration of Au^{3+} ions is no longer dependent on the dissociation of the chloroaurate ion, but on the dissociation of the cyanoaurate ion, which has been shown to be a much more stable complex than the chloroaurate ion. The resultant sudden drop in the concentration of Au^{3+} ions is reflected in the observed sudden change in potential according to the familiar Nernst equation. The potentials exhibited by the gold and platinum electrodes are a measure of the tendency of these Au^{3+} ions to loose their charge and deposit as a metallic film. As the material of the electrode does not take part in this process, it is evident that the gold and platinum electrodes should acquire the same potential in the same solution, as has been found experimentally during the course of the titration of chloroauric acid with potassium cyanide up to the equivalence point (ab in curves 1 and 3, Fig. 5).

However, the first excess of cyanide after the equivalence point exerts a corrosive effect on the gold electrode. This effect is, of course, also apparent when hydrochloric acid is titrated with potassium cyanide. This overshadows the effect due to the minute quantity of Au^{3+} ions resulting from the dissociation of the cyanoaurate complex, accordingly the function of a gold electrode in a solution containing excess cyanide is not materially affected by the presence or absence of the products of reaction of chloroauric acid and potassium cyanide (de in curves 1 and 4, Fig. 5).

Alkaline solution: spontaneous electrolysis. The pH value of a solution of 0·01M chloroauric acid, titrated to the electrometric equivalence point with potassium cyanide, was found to be 8·44. The aim of the following investigation in alkaline solution was to determine the effect of excess alkali on the reaction, in which case the trivalent gold would be in the form of the aurate AuO_2^- ion[6].

Baker and Morrison[7] determined microgram quantities of cyanide in alkaline solution by integrating the current generated when electrodes of platinum and silver were immersed in the solution over an arbitrary fixed time interval. In the present investigation a gold electrode was substituted for the silver in order to avoid replacement effects when solutions of gold compounds were used.

Spontaneous electrolysis apparatus. The plain end of a standard B24 socket was closed to form a flat-bottomed tube. The electrodes, sealed into glass tubes, were cemented into a B24 cone with plaster of Paris. The electrodes themselves consisted of 7 cm of gold wire of 0·6 mm diameter and 5 cm of platinum wire of the same diameter. The gold wire was coiled into a helix of 5 mm diameter and 1·5 mm pitch. The platinum electrode was in the form of a helix of 2 mm diameter and the same pitch, situated coaxially with respect to the gold electrode. The solution in the cell was kept stirred by a polythene-covered magnet driven by a synchronous motor at 520 revolutions per minute. The cell was enclosed in a thermostatically-controlled water jacket at 33·9°C. The external circuit comprised a microammeter with a range of 20 microamp connected across the electrodes.

The stoicheiometric relation between trivalent gold and potassium cyanide in strongly alkaline solution. Preliminary tests showed that the current efficiency fell far short of 100 per cent, thus precluding application of the theoretical considerations of Lingane[8, 9]. Accordingly it was not possible to determine the excess cyanide present in an alkaline solution containing trivalent gold. However, if the factors governing the current efficiency (temperature; alkali concentration; gold concentration; electrode size, condition and spacing; speed of stirring and external resistance) are fixed and only the cyanide concentration is varied, then the equivalence point should appear as a sharp change of direction of the graph of integrated current plotted against concentration of cyanide.

Procedure adopted for spontaneous electrolysis. To twice the required volume of standard potassium cyanide solution in a 10 ml volumetric flask was added 1N sodium hydroxide (2 ml). After diluting to 10 ml, 5 ml was transferred to the electrolytic cell and the stirrer set in motion. To this was added standard 0·01M chloroauric acid (5 ml). When the solution had reached the controlled temperature, the electrodes, previously cleaned in 1:1 hydrochloric acid followed by distilled water, were dropped into place. Current readings were then taken at suitable intervals and plotted against time, the area under the curve giving the integrated current or quantity of electricity. By plotting the molecular ratio of $KCN:HAuCl_4$ in each test solution against the corresponding integrated current produced, the curve in Fig. 6 was obtained, indicating that under these conditions an atom of gold is associated with six cyanide ions, suggesting the existence of an acid-labile trivalent anion analogous to ferricyanide, viz. $(Au(CN)_6)^{3-}$.

Under these conditions the pH value of the solution was 11·6₄.

Ammoniacal solution: complexometric titration. Adopting a technique described by Kinnunen and Merikanto,[10] the following procedure was employed for determining the cyanide equivalent of trivalent gold in ammoniacal solution of pH 9·75.

0·00745M Chloroauric acid (10 ml) was treated, in order, with 0·1N hydrochloric acid (5 ml), 2·5 per cent potassium nickelocyanide solution, $K_2Ni(CN)_4$, (10 ml), 10 ml of ammonium chloride-ammonium hydroxide buffer (350 ml concentrated ammonia and 54 g ammonium chloride per litre: pH 9·75) and 20 ml of 0·01M "EDTA" (disodium salt of ethylene diamine tetra-acetic acid). After 15 minutes 2 per cent ascorbic acid (5 ml) was added and the mixture titrated with 0·01M manganous sulphate using Eriochrome black T indicator. A blank titration was carried out in the absence of chloroauric acid. Satisfactory end points were obtained.

The difference between the two manganous sulphate titres amounted to 0·0925 millimoles, which is equivalent to 0·0925 millimoles of "EDTA" or four times 0·0925 millimoles of potassium cyanide, according to the following equations:

$$NiSO_4 + X^{2-} \longrightarrow NiX + SO_4{}^{2-}$$
$$NiSO_4 + 4KCN \longrightarrow K_2SO_4 + K_2Ni(CN)_4$$
(X^{2-} refers to the "EDTA" ion)

This amount of cyanide was taken up by 0·0745 millimoles of chloroauric acid. The resultant cyanide to gold ratio was 4·97, which may be taken as 5.

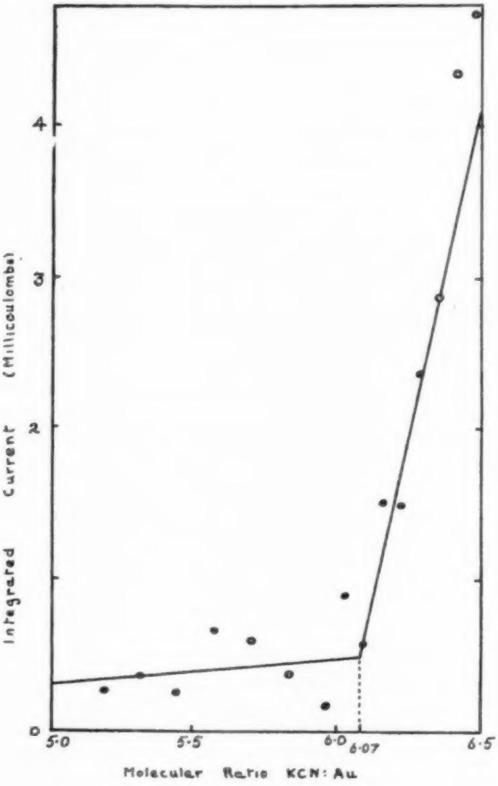


FIG. 6—Spontaneous electrolysis

DISCUSSION

The large change in potential of a gold electrode while immersed in a solution of chloroauric acid undergoing titration with potassium cyanide appears to be due to two reactions:

(i) $HAuCl_4 + 5KCN \longrightarrow KAu(CN)_4 + HCN + 4KCl$

(ii) $Au^\circ + 2CN^- \rightleftharpoons Au(CN)_2 + e.$

Following the completion of (i) which resulted in a drop in potential due to the decrease in concentration of Au^{3+}, further addition of potassium cyanide brought about reaction (ii) which caused a further lowering of the potential of the gold electrode.

In neutral or acid solution the trivalent gold cation is associated with four cyanide ions. This being so, the following monovalent complex anion suggests itself, viz., $(Au(CN)_4)^-$.

In ammoniacal solution of pH 9·75, the trivalent gold cation is associated with five cyanide ions, while in alkaline solution of pH 11·6 the trivalent gold cation is associated with six cyanide ions, suggesting the possibility of a trivalent complex anion, $(Au(CN)_6)^{3-}$.

Department of Chemistry,
University of the Witwatersrand,
Johannesburg.

Received November 17, 1959.

REFERENCES

[1] Murray, M.Sc. Thesis (University of the Witwatersrand), 1933.
[2] Raines, *Compt. rend. Acad. Sci. U.R.S.S.*, 1938, **18**, 339.
[3] Sneed, Maynard and Brasted, *Comprehensive Inorganic Chemistry*, D. van Nostrand Co. Inc., New York, 1954, p. 218.
[4] Conway, *Electrochemical Data*, Elsevier Publishing Co., London, 1952, p. 293.
[5] Bjerrum, *Bull. soc. chim. Belges.*, 1948, **57**, 432.
[6] Britton and Dodd, *J. Chem. Soc.*, 1932, 2464.
[7] Baker and Morrison, *Anal. Chem.*, 1955, **27**, 1306.
[8] Lingane, *Electroanalytical Chemistry*, Interscience Publishing Co., New York, 1953, p.194.
[9] Lingane, *Anal. Chim. Acta.*, 1948, **2**, 584.
[10] Kinnunen and Merikanto, *Chem. Analyst*, 1955, **44**, 11.

SOME PROPERTIES OF NITROCOTTON-NITROGLYCERINE GELS

by

P. W. LINDER

OPSOMMING

Die sogenoemde semigelatienplofstowwe bestaan uit nitrogliserien, nitrokatoen en sekere ander bestanddele. Nitrokatoen is 'n besondere soort selluloosnitraat. 'n Jel ontstaan wanneer nitrokatoen by nitrogliserien gevoeg word deurdat die nitrokatoen uitdy en die nitrogliserien geabsorbeer word. Dit is welbekend dat die gehalte van nitrokatoen 'n belangrike uitwerking het op onder andere die waterweerstand van semigelatienplofstowwe en die uitsyfering van nitrogliserien uit die plofstof. Die nadelige uitwerking van sekere nitrokatoen eienskappe word toegeskryf aan hul invloed op die struktuur van die nitrokatoen-nitrogliserien jel. Ten einde die uitwerking van die gehalte van nitrokatoen op die eienskappe van die jel te probeer verstaan is die uitsyfering van die nitrogliserien uit semigelatienplofstowwe, wat verhaas is deur uitswaaiing, bestudeer. Die uitgeswaaide nitrogliserien is met tussenposes geweeg en die resultate verkry kom ooreen met die kinetika van drie gelyktydige verlope van die eerste orde. Sekere parameters kan van die resultate bereken word en daar word aangetoon dat daar 'n verhouding tussen die parameters en die gehalte van nitrokatoen bestaan.

SUMMARY

Those blasting explosives known as semi-gelatines are composed of nitroglycerine, nitrocotton and certain other ingredients. Nitrocotton is a particular form of cellulose nitrate. This substance swells in nitroglycerine and absorbs the liquid to form a gel. It is known that nitrocotton quality affects certain properties of semi-gelatines, such as water resistance and the tendency of the nitroglycerine to percolate during storage of the explosive. It is thought that this deleterious effect of nitrocotton quality arises because the physical properties of the gel are affected. In order to gain an insight into the effect of nitrocotton quality on the physical properties of gels, a study has been made of nitroglycerine percolation accelerated by centrifuging semi-gelatines. By weighing the nitroglycerine spun out, after measured time intervals, data are obtained which fit the kinetics of three parallel first order processes. Certain parameters may be calculated from the data and these are shown to be related to nitrocotton quality.

Nitroglycerine and, to a smaller, extent, cellulose nitrate are used in the manufacture of nearly all modern blasting explosives. Blasting gelatine,[1] invented by Alfred Nobel in 1877, consists almost entirely of nitroglycerine and cellulose nitrate, and although once considered the ideal explosive, is relatively costly and is now used only for special purposes. Most blasting operations today are carried out with mixtures containing nitroglycerine, oxygen-donating salts such as sodium or ammonium nitrate, carbonaceous fuels, and sometimes cellulose nitrate. The mixtures are considerably cheaper than blasting gelatine and have the additional advantage that the proportions of the ingredients can be altered to give the desired degree of power, velocity of detonation or sensitiveness, and a consistency suitable for efficient production of cartridged explosives. The blasting explosives in current use are of three main types, namely the non-waterproof powders which do not contain cellulose nitrate, the waterproof gelignites with a plastic consistency and the waterproof cohesive powders known as semi-gelatines.

This paper is concerned with the gelignites and the semi-gelatines, which acquire their water resistant properties from the incorporation of cellulose nitrate in the nitroglycerine. In the Explosives Industry, cellulose nitrate is produced by esterifying raw cotton and the product is called "nitrocotton". This has the property of swelling in nitroglycerine and absorbing the liquid to form a gel. In the production of gelignites and semi-gelatines, the gel is prepared first and gelation is usually facilitated by the addition of a small quantity of alcohol.

It is important that semi-gelatines have adequate water resistance and that they retain the nitroglycerine so that percolation does not occur during transportation and storage. The degree of water resistance of standard gelignite formulations is intrinsically high, but these explosives must be guaranteed not to exude nitroglycerine at any stage during transportation or storage. Experience has shown that these qualities are governed chiefly by the quality of the nitrocotton. Since nitrocotton is manufactured from natural cotton, the quality is not always consistent and, therefore, it is necessary to subject this ingredient to rigorous tests.

A number of empirical tests aimed at evaluating the quality of nitrocotton are in current use but the measurements made are difficult to interpret because it is not known which physical properties of nitrocotton govern the quality. Instead of carrying out tests on nitrocotton itself, it is often the practice to prepare one or more semi-gelatines and one or more gelignites and to determine the water resistance and percolation tendency of the semi-gelatines and the exudation tendency of the gelignites.

In order to determine the water resistance of a semi-gelatine a number of 1″ × 8″ cartridges are immersed in water. Before immersing the cartridges, seven helically distributed radial holes are pierced in each. After definite periods of immersion groups of three to ten cartridges are removed from the water and are tested by determining whether or not they can be detonated using a standard detonator. It is preferable to treat the results by a statistical method[2] and to express the water resistance as that time of immersion after which the probability of detonation is 50%. The disadvantages of this method are that it constitutes a "go no go" test; in order to obtain a high precision the number of cartridges required is impracticably high, and the result can be expressed only in terms of a probability.

The percolation tendency of a semi-gelatine may be determined by storing cartridges in vertical positions for a definite period of time. The concentration gradient of the nitroglycerine along the length of a cartridge is then determined. The flow of nitroglycerine is slow so that this method is time-consuming. The author[3] has developed a rapid test for determining the percolation tendency of a semigelatine in which the explosive is centrifuged at 922 G for fifteen minutes. In this test the mass of nitroglycerine spun out of the explosive is used as an index of the tendency to percolate. This method involves an accurate measurement and the precision is reasonably high.

The exudation tendency of a gelignite may be measured by storing cartridges of this explosive for prolonged periods, at elevated temperatures or under a reduced ambient pressure. It is customary to use the "vacuum exudation test"[4] in which ten cartridges are allowed to stand under a pressure of 30 cm mercury (absolute) for 24 hours. The amount of nitroglycerine which has exuded is then estimated by inspection and this amount indicates the exudation tendency. The estimate is expressed as an integer on a scale from 0 to 10, 0 corresponding to zero exudation. The exudation tendency of the batch of gelignite is then expressed as the sum of the exudation values of the ten cartridges tested. The disadvantage of this method is that the estimation is subjective.

None of the tests referred to above throw any light on the reason why nitrocotton quality governs the water resistance and percolation tendency of semi-gelatines and the exudation tendency of gelignites. It is reasonable to suppose that the effect of nitrocotton quality would be to govern the physical properties of the nitrocotton-nitroglycerine gel, which in turn would affect the above mentioned properties of the explosive. A nitrocotton-nitroglycerine gel might reasonably be expected to contain both bound and free nitroglycerine. It should therefore be possible to express the degree of gelation by the relative amounts of bound or free nitroglycerine. Furthermore, if a sample of an explosive which contains a nitrocotton-nitroglycerine gel were centrifuged as in the percolation test mentioned, the rates of percolation of the bound nitroglycerine and the free nitroglycerine would be different. Hence, a study of the kinetics of the process would allow a distinction to be made between the two. In order to investigate whether there are distinct types of binding of the nitroglycerine in a nitrocotton-nitroglycerine gel a study has been made of the kinetics of centrifugal percolation of nitroglycerine in semi-gelatine.

Originally it was assumed that the gel in a semi-gelatine contained only the two types of nitroglycerine mentioned, namely, bound and free. Preliminary experiments indicated, however, that the kinetic data could be fitted better to a theory based on the assumption that there may be up to three degrees of binding of the nitroglycerine.

THEORY

It is assumed that in a nitrocotton-nitroglycerine gel an amount Nb of the nitroglycerine is strongly bound, an amount Nl is loosely bound and an amount Nf is free.

If the total amount of nitroglycerine is N_o, it follows that

$$N_o = Nb_o + Nl_o + Nf_o \quad \ldots \quad (1)$$

where the subscript $_o$ refers to the initial amounts of nitroglycerine before centrifuging.

During centrifuging all three types of nitroglycerine percolate from the semi-gelatine to give an amount Ns of spun out nitroglycerine. Analysis of experimental Ns values determined as a function of time enable Nb_o, Nl_o and Nf_o to be evaluated. The analysis is analagous to that of Brown and Fletcher[5] in the treatment of results obtained for the hydrolysis of mixed tertiary aliphatic chlorides.

It is reasonable to suppose that the three percolation processes are kinetically of the first order. Accordingly the rate equations are

$$-\frac{dNb}{dt} = k_bNb \quad \ldots \quad (2)$$

$$-\frac{dNl}{dt} = k_lNl \quad \ldots \quad (3)$$

$$-\frac{dNf}{dt} = k_fNf \quad \ldots \quad (4)$$

where k_b, k_l and k_f are the respective rate constants.

Integration of equations (2), (3) and (4) yields

$$Nb = Nb_oe^{-k_bt} \quad \ldots \quad (5)$$

$$Nl = N_oe^{-k_lt} \quad \ldots \quad (6)$$

$$Nf = Nf_oe^{-k_ft} \quad \ldots \quad (7)$$

Since

$$Ns = N_o - (Nb + Nl + Nf) \quad \ldots \ldots \ldots \quad (8)$$

it follows that

$$Ns = N_o - (Nb_o e^{-k_b t} + Nl_o e^{-k_l t} + Nf_o e^{-k_f t}) \quad \ldots \ldots \quad (9)$$

and

$$\ln(N_o - Ns) = \ln(Nb_o e^{-k_b t} + Nl_o e^{-k_l t} + Nf_o e^{-k_f t}) \quad \ldots \ldots \quad (10$$

If it is assumed that

$$k_f \gg k_l \gg k_b,$$

after a certain time, Nl and Nf become effectively zero, so that equation (10) reduces to

$$\ln(N_o - Ns) = \ln Nb_o - k_b t \quad \ldots \ldots \ldots \quad (11)$$
$$= \ln Nb$$

Thus the expression for $\ln(N_o - Ns)$ becomes linear. From the slope and intercept of the straight line, k_b and Nb_o may be determined and Nb calculated for every value of t.

Since

$$Nl + Nf = N_o - Ns - Nb \quad \ldots \ldots \ldots \quad (12)$$

it follows that

$$\ln [(N_o - Ns) - N_b] = \ln(Nl_o e^{-k_l t} + Nf_o e^{-k_f t}) \quad \ldots \ldots \quad (13)$$

and because it is assumed that

$$k_f \gg k_l$$

after a certain time, Nf becomes effectively zero, so that equation (13) reduces to

$$\ln [(N_o - Ns) - N_b] = \ln Nl_o - k_l t \quad \ldots \ldots \ldots \quad (14)$$
$$= \ln Nl$$

Thus the expression of $\ln [(N_o - Ns) - N_b]$ becomes linear. From the slope and intercept of the straight line k_l and Nl_o may be determined and Nl calculated for every value of t.

Finally, Nf may be determined for every value of t, since, from equation (8)

$$Nf = (N_o - Ns) - Nb - Nl \quad \ldots \ldots \ldots \quad (15)$$

Alternatively, Nf_o may be found directly by substituting the determined values of Nb_o and Nl_o in equation (1).

EXPERIMENTAL

Preparation of explosives. Fourteen different nitrocotton batches covering a range of qualities were used to prepare semi-gelatines according to standard procedures. Semi-gelatines were prepared in triplicate from each of three of the nitrocottons, and duplicate semi-gelatines were made from one of the nitrocottons. In all, therefore, twenty-one semi-gelatines were prepared.

Determination of Nb_o, Nl_o and Nf_o for the semi-gelatines. Quadruplicate 50 g quantities of each semi-gelatine were placed in the buckets illustrated in Fig. 1. These were then centrifuged at 922 G for a total of approximately 200 minutes in accurately timed ten minute increments. At the end of each ten minute interval, the centrifuge was stopped and the spun out nitroglycerine weighed. These intervals were made of equal duration so that at each datum point a constant error due to the acceleration and deceleration of the centrifuge was imposed on the time co-ordinate.

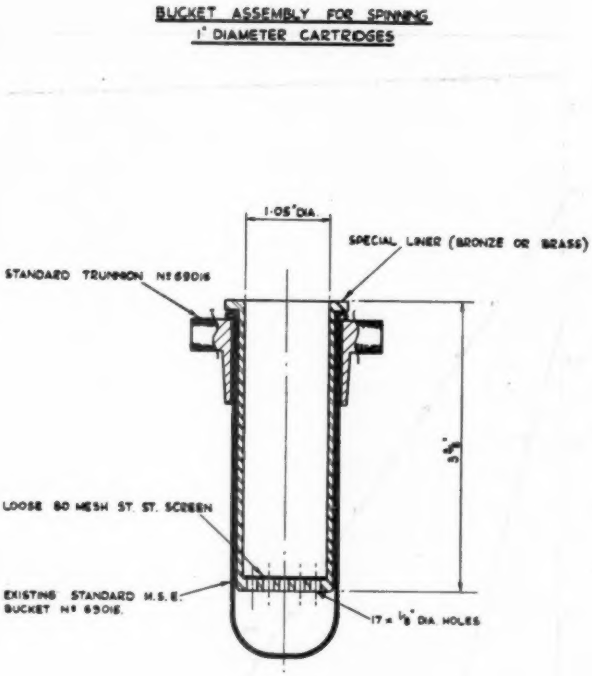

BUCKET ASSEMBLY FOR SPINNING
1" DIAMETER CARTRIDGES
1·05" DIA.
SPECIAL LINER (BRONZE OR BRASS)
STANDARD TRUNNION Nº 69016
3½"
LOOSE 80 MESH ST. ST. SCREEN
EXISTING STANDARD M.S.E. BUCKET Nº 69015.
17 × ⅛" DIA. HOLES

Fig. 1

Since absolutely uniform mixing could not be achieved the nitroglycerine content of the 50 g test specimens may have varied slightly from one to another and so N_0 may not have been known precisely. Consequently, at the end of a run, each residual specimen was extracted in a Wiley apparatus[6] with methylene chloride and the nitroglycerine in the extract determined by Becker's[7] method. For any specimen N_0 was then given by the sum of the nitroglycerine in the residue and the total amount of nitroglycerine spun out.

RESULTS

For each time, the quadruplicate (N_0 — Ns) values obtained for each of the 21 semi-gelatines were summed, yielding a set of data to which the theoretical treatment was applied and values of Nb_0, Nl_0 and Nf_0 calculated.

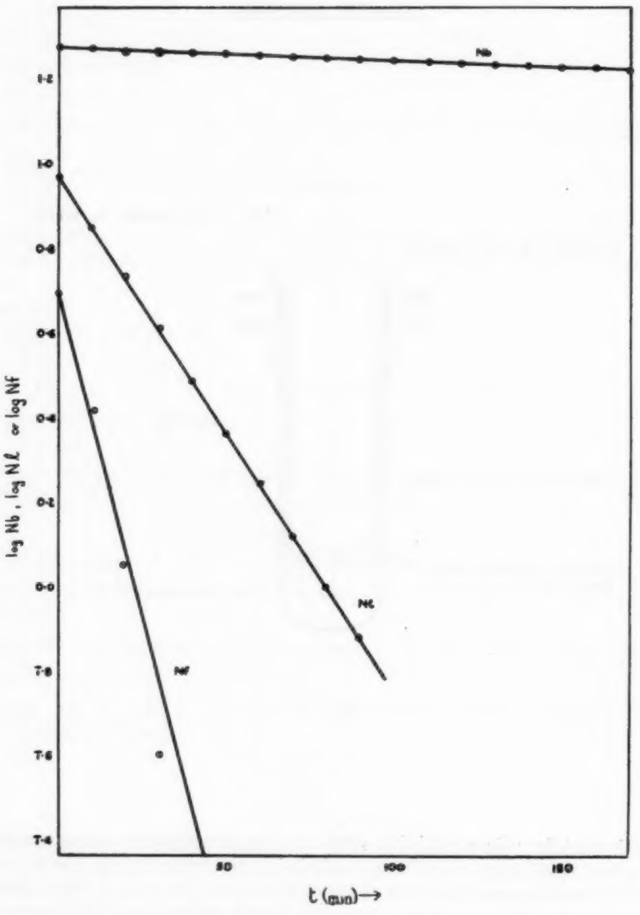


Fig. 2—Parameters for nitrocotton No. 6

Fig. 2 shows separate plots of the values of log Nb, log Nl and log Nf actually obtained for a semi-gelatine made from a nitrocotton of poor quality. In general, the theory was found to fit the centrifugal percolation rate data satisfactorily, and values of Nb_0, Nl_0 and Nf_0 could be calculated with confidence.

The complete set of determinations, showing values obtained for Nb_O, Nl_O and Nf_O, is shown in Table I.

TABLE I

Values of Nb_o, Nl_o and Nf_o for 21 semi-gelatines

Nitrocotton	Nb_o %	Nl_o %	Nf_o %
1	100	0	0
	100	0	0
	99·5	0·5	0
2	100	0	0
	100	0	0
3	95·7	4·3	0
4	67·8	32·2	0
5	61·3	27·7	11·0
6	56·8	28·4	14·8
7	54·4	27·6	18·0
8	52·1	35·4	12·5
9	54·0	25·5	20·5
	59·6	28·9	11·6
	50·7	28·6	20·7
10	43·8	24·0	32·2
11	43·3	28·5	28·2
12	52·6	21·4	26·0
	39·7	21·7	38·6
	42·7	26·3	31·0
13	37·2	26·2	36·6
14	34·9	20·9	44·2

In Fig. 3 the values are plotted on triangular co-ordinates. The points appear to lie on two intersecting straight lines, one joining the Nb_O apex to the point ($Nf_O = O$, $Nl_O \doteqdot 34\%$) and the other spanning the triangle obliquely from the latter towards the point ($Nf_O \doteqdot 90\%$, $Nl_O \doteqdot 10\%$).

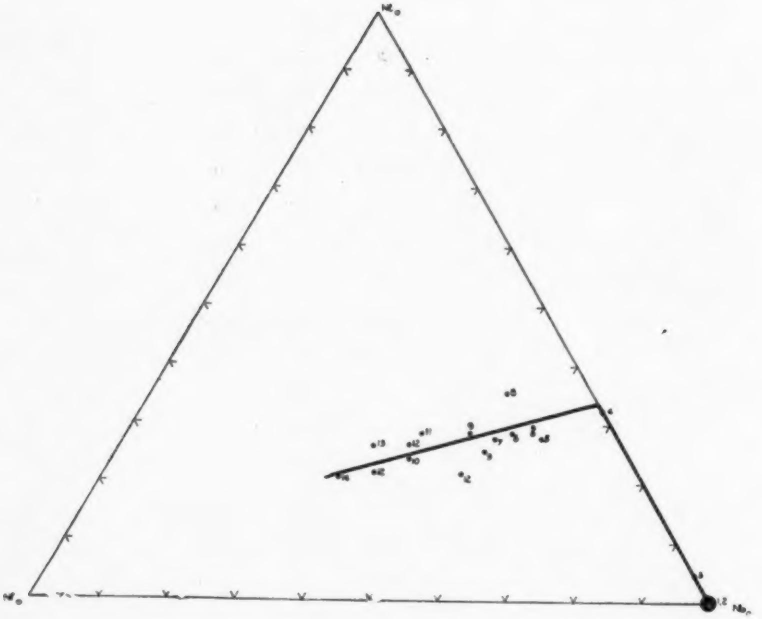


Fig. 3—Triangular curve for values of Nb_o, Nl_o and Nf_o obtained for the twenty-one semi-gelatines

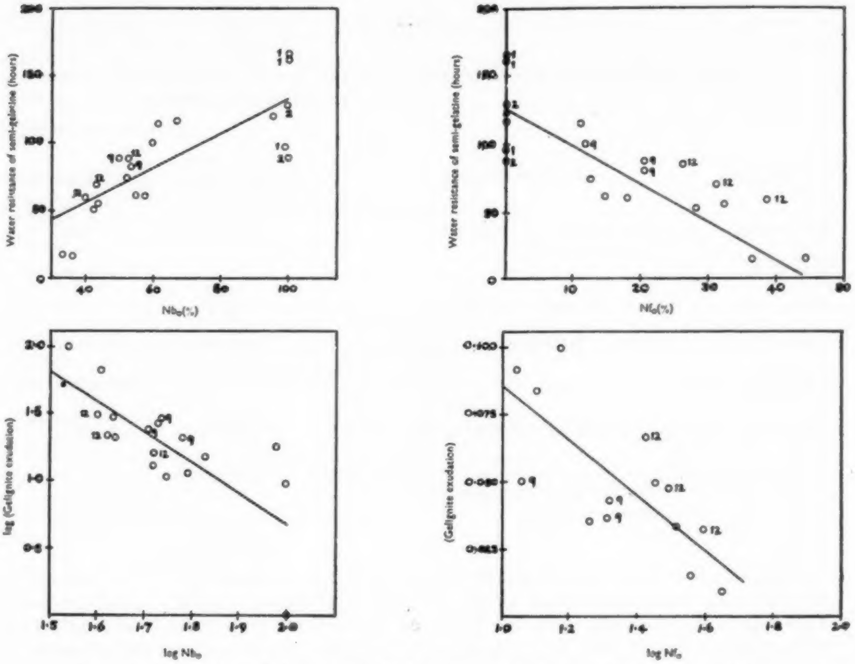


Fig. 4—Correlations betweet percolation parameters and explosive properties. The lines represent the calculated regression equations.

DISCUSSION

The centrifugal percolation rate data for the 21 semi-gelatines examined can be represented consistently by the rate theory proposed. This supports the assumption that there may be up to three discrete degrees of binding in a nitrocotton-nitroglycerine gel from which a semi-gelatine is made. Amongst the nitrocotton batches studied the quality of some was so high that a value for Nb_0 of 100% was obtained. In contrast, high values of Nf_0 were obtained for other nitrocotton batches. Between the two extremes, for certain of the nitrocotton batches, positive values were obtained for Nb_0 and Nl_0 whilst Nf_0 was found to be zero.

In order to illustrate that the parameters Nb_0, Nl_0 and Nf_0 are related to nitrocotton quality they were compared with the water resistance of the semi-gelatines. In addition, these parameters were compared with the exudation tendencies of gelignites made from the same fourteen nitrocotton batches. The details of the comparisons and the results of a statistical analysis are given in Table II. Fig. 4 illustrates graphically the calculated regression lines and shows plots of the experimental results. It can be seen that highly significant correlations were found between

Nb_0 and semi-gelatine water resistance,
Nf_0 and semi-gelatine water resistance,
Nb_0 and gelignite exudation,
and Nf_0 and gelignite exudation.

TABLE II

Correlations between the percolation parameters and (1) *semi-gelatine water resistance*
(2) *gelignite exudation*

Variables	Regression equation	Correlation coefficient, r	Significance level of r, %
WR vs Nb_o	$WR = 4{\cdot}0799 + 1{\cdot}2855\ Nb_o$	0·7908	0·1
WR vs Nl_o	—	—	not significant
WR vs Nf_o	$WR = 123{\cdot}8097 - 2{\cdot}2090\ Nf_o$	—0·8259	0·1
Ex vs Nb_o	$\log(Ex) = 5{\cdot}1667 - 2{\cdot}2473 \log Nb_o$	—0·7593	0·1
Ex vs Nl_o	—	—	not significant
Ex vs Nf_o	$\frac{1}{Ex} = 0{\cdot}1836 - 0{\cdot}09895 \log Nf_o$	—0·7504	1

WR = semi-gelatine water resistance
Ex = gelignite exudation

Although several transformations were tried no correlation between either the semi-gelatine water resistance or the gelignite exudation and Nl_O could be found. This was not surprising since the value of Nl_O did not vary greatly amongst those semi-gelatines for which $Nf_O > O$. At any rate, those correlations which were found provide strong evidence that the parameters Nb_O and Nf_O are related to nitrocotton quality.

It would not be wise to assume that Nb_O and Nf_O are exact quantitative measures of nitrocotton quality. The reason for this is that although the precision in determining Nb_O, Nl_O and Nf_O for a single semi-gelatine was found to be high, the reproducibility in each of the two triplicate sets of results for nitrocotton number 9 and number 12 was poor. This variation amongst the member semi-gelatines of a triplicate set made from a single nitrocotton may be attributed to the influence of other factors such as the conditions obtaining during the preparation of the explosives. Hence, Nb_O and Nf_O can be regarded as giving only an approximate evaluation of nitrocotton quality. The approximation, however, is no worse than that of using semi-gelatine water resistance or gelignite exudation values as measures of nitrocotton quality, since these properties also vary amongst batches of the respective explosives prepared with any one nitrocotton batch. That the poor reproducibility of Nb_O, Nl_O and Nf_O is due to the same cause as the variation in the properties of replicated semi-gelatines or gelignites is shown by the fact that within either of the two triplicated observations, the variations of water resistance or of exudation with Nb_O and Nf_O are in line with the overall correlations involving all the results.

Thus, in addition to giving some insight into the physical meaning of nitrocotton quality, this kinetic centrifugal method provides a superior means of estimating nitrocotton quality. Whilst the approximation involved in the estimation is no worse than in the other methods mentioned, this method has the advantage of being less time-consuming and more precise than the determination of water resistance. Furthermore, the results are not expressed in terms of probabilities.

The author expresses his sincere thanks to Drs. F. L. Clark, H. A. E. Mackenzie and L. H. Stein for extremely helpful discussions and encouragement, to his colleague Dr. G. S. Harrison for discussions and active assistance with calculations and to Messrs. African Explosives and Chemical Industries, Limited, for permission to publish this paper.

Research Department,
African Explosives and Chemical Industries, Limited,
P.O. Northrand,
Transvaal.

Received December 17, 1959.

REFERENCES

[1] Taylor, *Detonation in Condensed Explosives*, Clarendon Press, Oxford, 1952, p. 14.
[2] Finney, *Probit Analysis*, Cambridge, 1952, p. 226.
[3] Linder, *African Explosives and Chemical Industries, Ltd.*, internal report, 1959, unpublished work.
[4] Malan, *African Explosives and Chemical Industries, Ltd.*, internal report, 1936, unpublished work.
[5] Brown and Fletcher, *J. Am. Chem. Soc.*, 1949, **71**, 1845.
[6] Strouts, Gilfillan and Wilson, *Analytical Chemistry. The Working Tools*, Clarendon Press, Oxford, 1955, p. 25.
[7] Becker, *Ind. Eng. Chem. Anal.*, 1933, **5**, 152.

NOTES

STYRENE-*P*-CARBOXYLIC ACID

by

L. DYKSTERHUIS and D. E. A. RIVETT

Styrene-*p*-carboxylic acid was first prepared by Marvel and Overberger in 1945. Recently several alternative syntheses [2, 3, 4, 5] have been published. We were unsuccessful with Bergmann and Blum's method [4] since permanganate oxidation of *p*-methylacetophenone in our hands gave only terephthalic acid and no *p*-acetylbenzoic acid, the required starting material.

We have modified Cazes' method [2] by employing potassium borohydride (instead of aluminium isopropoxide) reduction of *p*-bromoacetophenone. The resulting carbinol was dehydrated with potassium bisulphate, instead of with phosphorus pentoxide in boiling benzene, to *p*-bromostyrene, which was converted without purification via the Grignard reagent in tetrahydrofuran to styrene-*p*-carboxylic acid. The overall yield of 42% is better than that recorded by any previous method. Some new derivatives of styrene-*p*-carboxylic acid are recorded.

EXPERIMENTAL

All melting points are corrected and were obtained on a Kofler block.

p-(α-Hydroxyethyl)bromobenzene. Potassium borohydride (18·3 g, 0·34 mole) was added with continuous stirring in 1-2 g portions to a solution of *p*-bromoacetophenone (228 g, 1·15 mole) in methanol (600 ml). The solution became hot and was kept below boiling point by periodical cooling. After stirring a further half hour the solution was acidified with hydrochloric acid, most of the methanol removed under reduced pressure, water added and the mixture extracted with ether. The product (205 g, 89%) distilled at 124°/3 mm or 82°/0·3 mm (lit. [7, 8] b.p. 130°/12 mm, 133°/15 mm) and had N_D^{27} 1·565 (lit. [7, 8] N_D^{18} 1·574, N_D^{20} 1·5697). It crystallised on cooling to -10° and melted at 20-30°; this substance has not been obtained crystalline before.

The phenylurethane melted at 103-4° (lit. [7] m.p. 103-4°).

p-Bromostyrene. A 100 ml Claisen flask was half filled with finely powdered anhydrous potassium bisulphate and heated at 210-230° in a vaccum of 20 mm. *p*-(α-Hydroxyethyl)bromobenzene (204 g) was added dropwise through a dropping funnel (tip inside the bisulphate) at such a speed that the distillation temperature did not exceed 100°. The distillate was dried over sodium sulphate; yield 156 g (84%). It distilled at 97°/20 mm (lit. [8] b.p. 88°5-89·5°/16 mm) with much loss through polymerisation.

Styrene-p-carboxylic acid. This was prepared by Cazes' method from crude *p*-bromostyrene in 56% yield, after crystallisation from 20% aqueous ethanol, and had m.p. 136-138° (lit. [1, 2] 143-4°, 136°). Redistilled *p*-bromostyrene gave a 77% yield of the acid.

The anilide, after crystallisation from methanol, formed colourless needles, m.p. 170-172° (Found: C, 80·4; H, 5·9. Calc. for $C_{15}H_{13}ON$: C, 80·7; H, 5·9%).

p-(α,β-Dibromoethyl)benzoic acid. A solution of bromine (1·0 g) in carbon tetrachloride (10 ml) was added dropwise to styrene-*p*-carboxylic acid (1·0 g) dissolved in the same solvent. After half an hour the crystalline precipitate (1·4 g) was separated and sublimed at 130°/0·5 mm, m.p. 168° after darkening at 160° (Found: C, 34·9; H, 2·7. Calc. for $C_9H_8O_2Br_2$: C, 35·0; H, 2·6%).

The acid was converted to the methyl ester with dry hydrobromic acid in methanol, m.p. 76° after crystallisation from aqueous methanol (Found: C, 37·3; H, 3·0. Calc. for $C_{10}H_{10}0_2Br_2$: C, 37·3; H, 3·1%).

The anilide, prepared in the usual way, crystallised from methanol in long colourless needles, m.p. 205° (Found: C, 47·2; H, 3·3. Calc. for $C_{15}H_{13}0Br_2N$: C, 47·0; H, 3·4%).

p-(α,β-Dithiocyanoethyl)benzoic acid. A mixture of styrene-*p*-carboxylic acid (1·0 g), potassium thiocyanate (6 g), anhydrous copper sulphate (6 g) and glacial acetic acid (15 ml) was left overnight at 5°[9]. Water was added and the product extracted with benzene. It crystallised from benzene in thick colourless needles, m.p. 170-1° (Found: C, 50·0: H, 3·0. Calc. for $C_{11}H_8 0_2S_2N_2$: C, 50·0; H, 3·0%).

p-(α-Chloro-β-p-methylthiophenyl)benzoic acid. A solution of *p*-methylsulphenyl chloride[10] (1 g) and styrene-*p*-carboxylic acid (0·5 g) in glacial acetic acid (10 ml) was left overnight and a few drops of water then added. The resulting crystalline precipitate was recrystallised from benzene-hexane mixture and then from benzene, m.p. 171° (Found: C, 62·2; H, 5·1. Calc. for $C_{16}H_{15}0_2$ Cl S: C, 62·7, H, 4·9%).

p-(α-Chloro-β-2′,4′-dinitrothiophenylethyl)benzoic acid. A solution of 2,4-dinitrosulphenyl chloride[11] (1·0 g) and styrene-*p*-carboxylic acid (0·5 g) in glacial acetic acid (15 ml) was heated on the steambath for two hours and allowed to stand overnight. The resulting crystalline precipitate (0·75 g) was recrystallised twice from acetic acid, m.p. 203-4° (Found: C, 47·3; H, 3·0. Calc. for $C_{15}H_{11}0_6N_2$Cl S: C, 47·1; H, 2·9%).

We thank the ISCOR Research Laboratory and the National Chemical Research Laboratory, Pretoria, for the microanalyses.

Agricura Laboratoria Limited,
P.O. Box 55,
Silverton.

Received January 24, 1960.

REFERENCES

[1] Marvel and Overberger, *J. Am. Chem. Soc.*, 1945, **67**, 2250. See also Emerson, Heyd, Lucas, Chapin, Owen and Shortridge, *ibid.*, 1946, **68**, 674.
[2] Cazes, *Comp. rend.*, 1958, **247**, 1874.
[3] Kolensnikov and Soboleva, *Izvest. Akad. Nauk S.S.S.R. otdel Khim. Nauk.*, 1958, 762; *Chem. Abstr.* 1958, **52**, 20025.
[4] Bergmann and Blum, *J. Org. Chem.*, 1959, **24**, 549.
[5] Bissell and Spenger, *ibid.*, 1959, **24**, 1146.
[6] *Org. Synthesis, Coll. Vol. I*, 1941, 109.
[7] Quelet, *Bull. soc. chim. France*, 1929, **45**, 86.
[8] Ziegler and Tiemann, *Ber.*, 1922, **55**, 3414.
[9] Dermer and Dysinger, *J. Am. Chem. Soc.*, 1939, **61**, 750.
[10] Lecher, Holschneider, Koberle, Speer and Stocklin, *Ber.*, 1925, **58**, 409.
[11] Perold and Snyman, *J. Am. Chem. Soc.*, 1951, **73**, 2379.

PRODUCTION OF FAT BY *POLYPORUS RUGULOSUS*

by

JACK ADLER

Polyporus regulosus (T.R.L. 25), is a white rot wood-destroying fungus prevalent in the gold mines of the Witwatersrand, and causes much loss of timber underground if destruction of the latter is not retarded or prevented by treatment with preservatives. On account of the economic importance of the fungus the metabolism of the organism is under investigation. Among the metabolic products formed by this fungus are fatty materials, with which this communication deals.

Polyporus regulosus was grown on the surface of a medium consisting of 2 per cent malt and 5 per cent glucose, in a constant temperature room at 27·2°C and 75% relative humidity. Harvesting of mycelium was started after 24 days' growth because it was only produced in significant amounts after this period.

The mycelium was filtered off and washed twice with warm water. The filtrate and washings were combined and the total sugars estimated by the method of Somogyi.[1] The mycelium was dried to constant weight at 60°C and weighed. The fat was extracted with ether from the mycelium after preliminary hydrolysis with 2% hydrochloric acid on a steam bath for 2 hours. The solvent-free fatty material was weighed and the acid value, saponification value, and unsaponifiable matter determined successively on the same sample.

The results obtained are summarised in the accompanying Table.

DISCUSSION

After 30 days growth the conversion coefficient of glucose to mycelium becomes practically constant (0·2 ± 0·013 g mycelium/g glucose used). The fungus produces fat slowly and in relatively small amounts. The fat content of the mycelium remains constant for the first 46 days of growth (7·3 ± 2·6 g fat/100 g mycelium), then drops abruptly to about one third of this figure (2·5 ± 1·4 g fat/100 g mycelium) and remains constant at this value for the next 54 days.

The fat produced contains an average of about 8% unsaponifiable material. No ergosterol could be detected spectrophotometrically.

The free fatty acid content of the fat is high, but there is clearly a significant downward trend during the first 54 days of growth, indicating that glycerol synthesis is at a maximum only in older mycelium. Although there is a large variation in the molecular weight of these fatty acids, there is some indication that their molecular weight increases with time.

EXPERIMENTAL

Fat and fatty acid production by P. Rugulosus

Days of growth	G mycelium per flask	G mycelium per g glucose used	G fat per 100 g mycelium	% Free acid as oleic acid	% Un-sap.	M.W.
24	0·15	0·50	8·0	66·6	9	243
26	0·18	1·80	6·7	71·1	5	209
28	0·21	0·53	6·7	66·4	13	196
30	0·24	0·30	6·7	69·5	4	206
32	0·46	0·19	8·7	49·5	17	234
34	0·76	0·20	12·2	41·6	13	308
36	0·81	0·19	6·0	51·8	16	249
38	0·86	0·20	5·9	50·0	4	291
40	0·84	0·19	6·3	39·0	9	327
42	0·84	0·18	6·2	44·3	5	281
44	0·98	0·16	8·4	39·7	6	313
46	1·10	0·16	3·9	42·4	3	224
48	1·37	0·18	2·9	42·6	6	284
50	1·19	0·25	1·9	30·1	7	299
52	1·59	0·20	1·5	42·6	4	231
54	1·62	0·21	2·3	42·3	7	295
56	1·68	0·20	3·2	49·9	7	290
58	1·84	0·19	2·0	54·5	5	246
60	2·34	0·22	2·6	44·0	8	329
62	2·51	0·26	1·5	42·5	4	334
64	2·42	0·21	2·4	32·1	7	267
66	1·85	0·19	2·8	47·8	9	210
68	2·74	0·27	1·4	52·4	5	305
70	1·98	0·21	2·6	42·7	7	293
72	2·21	0·22	2·1	25·1	13	290
74	2·31	0·17	4·4	23·1	5	394
76	2·68	0·20	2·9	—	19	—
78	2·33	0·19	2·5	33·9	11	321
80	2·79	0·22	1·8	35·5	10	265
85	3·03	0·22	1·7	37·5	7	256
90	3·08	0·21	1·2	33·3	5	238
95	2·10	0·15	3·4	35·5	9	319
100	2·48	0·17	3·1	35·5	14	381

1. Figures are in all cases averages of 2 sets of 2 flasks, except the first 4 figures which were averages of two sets of 6, 5, 4 and 3 flasks respectively.
2. Molecular weights were calculated from saponification values after allowance had been made for the unsaponifiable material and glycerol present.
3. M.W. = molecular weight of fatty acids (free and combined).

Biochemical and Chemical Research Laboratory,
Transvaal and Orange Free State Chamber of Mines,
Johannesburg.

Received February 3, 1960.

REFERENCE

[1] Somogyi, *J. Biol. Chem.*, 1945, **160**, 61.

VIR DIE AANDAG VAN OUTEURS

Die Joernaal van die Suid-Afrikaanse Chemiese Instituut word gewy aan die publikasie van oorspronklike navorsing in alle vertakkings van chemie en chemiese tegnologie.

Alle bydraes moet in triplikaat ingehandig word; indien daar grafieke is moet een stel noukeurig met *Oosindiese ink afgetrek word op gladde wit Bristol-blad of tekenpapier, of op aftrekdoek*—die ander twee stelle kan afdrukke wees, of met potlood geteken word.

Artikels moet so saaklik moontlik wees, verenigbaar met duidelikheid, en moet nie onnodige eksperimentele besonderhede bevat nie. Artikels moet *in tikskrif wees—dubbel spasiëring*—net aan een kant van die papier, met 'n behoorlike kantruimte aan die linkerkant. Die papier moet nie absorberend wees.

Die buitemate van plate—insluitende die ruimte vir byskrif—moet hoogstens 14 duim by 10 duim wees, en liefs nie minder as 7 duim by 5 duim nie. Die verhouding 1.4:1.0 moet in elk geval nagekom word. Byskrif en letters—hetsy in die kantruimte of in die tekening self—moet nie met ink gedoen word nie, *maar liggies met gewone potlood of uitveebare bloupotlood.*

Elke artikel moet voorafgegaan word deur 'n opsomming, normaalweg van 50 tot 250 woorde lank, in elk van die offisiële landstale. Die opsomming wat tweede verskyn, moet in dieselfde taal as die artikel wees.

Verwysings moet in numeriese opeenvolging aan die end verskyn, in die volgorde: skrywer se van; joernaal; datum; deel; bladsy; die ooreenstemmende verwysingsnommers moet in volgorde in die teks as kopskrifte verskyn. Verwysings na boeke moet soos volg aangedui word: skrywer se van; titel; uitgawe; uitgewer; datum van publikasie; bladsy.

Simbole en afkortings moet voorlopig analoog aan B.S. 1991:1954 wees, terwyl titels van joernale afgekort moet word ooreenkomstig *Chemical Abstracts*.

Outeurs word versoek om hulle bydraes so op te stel dat dit ooreenstem met die gewone trant, konvensie en kernagtigheid van hierdie joernaal.

Dui asseblief aan waarheen die drukproewe gestuur moet word.

Rig asseblief alle korrespondensie aan die Redakteur van die Joernaal, Suid-Afrikaanse Chemiese Instituut, Kelvinhuis, h/v Marshall- en Hollardstraat, Johannesburg.

NOTICE TO AUTHORS

The Journal of the South African Chemical Institute is designed for the publication of original work in all branches of chemistry and chemical technology.

All papers must be submitted in triplicate; where diagrams are included one set of these must be carefully drawn, *in Indian ink, on smooth white Bristol Board or paper, or on tracing cloth*, the remaining two copies may be pencil drawings or prints.

Papers should be as brief as possible compatible with clarity, and no unnecessary experimental details should be included. All papers must be *typewritten in double line spacing*, on one side only of the paper, with a good margin on the left-hand side. The paper should be non-absorbent.

The total external dimensions of illustrations (including space to be taken up by lettering) should not exceed 14 inches by 10 inches and preferably should not be less than 7 inches by 5 inches—the proportion 1.4:1.0 being in all cases maintained. Lettering on drawings, whether on the margin or in the body of the drawing must not be in ink but must be inserted *lightly, in blue pencil or erasable lead pencil.*

Every paper must be preceeded by two summaries, one in each official language and normally from 50 to 250 words in length. The second summary must be in the language of the paper.

References should appear at the end of the paper, in numerical order in the following sequence: author's surname; journal; date; volume number; page; the necessary reference numbers should appear, in sequence, as superscripts in the text. References to books should give: author's surname; title; edition; publisher; date of publication; page.

Symbols and abbreviations used should conform to B.S. 1991:1954. Abbreviations of titles of journals should follow *Chemical Abstracts*.

Authors are requested to arrange their papers to conform, as far as possible, to the normal style, conventions and conciseness of this journal.

The address to which proofs are to be sent should be written on every paper.

All communications should be addressed to the Editor of the Journal, The South African Chemical Institute, Kelvin House, cor. Marshall and Hollard Streets, Johannesburg.